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ABSTRACT

This instructor's manual presents material on basic bacteriological laboratory procedures as required by Federal Register Water Quality Guidelines. Course topics include: characteristics, occurrences, and significance of bacterial indicators of pollution; bacteriological water quality standards and criteria; collection and handling of samples; laboratory test procedures; and data analysis. The guide consists of two major parts. Part I contains information required for course planning and management. Part II consists of a series of worksheets which provide learning objectives and a suggested instructional approach for each topic included in the training manual. (CO)

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United States
Environmental Protection
Agency

National Training
and Operational
Technology Center
Cincinnati OH 45268

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September 1980

Water

EPA

ED209116

Bacteriological Methods in Water Quality Control Programs

Instructor's Guide

U.S. DEPARTMENT OF EDUCATION
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SE 035 925

INSTRUCTOR'S GUIDE

for

CONDUCTING THE COURSE

BACTERIOLOGICAL METHODS

IN

WATER QUALITY CONTROL PROGRAMS

National Training and Operational Technology Center
Office of Water Program Operations
U. S. Environmental Protection Agency

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Preface

Introduction

This is one of several courses offered by the National Training and Operational Technology Center (NTOTC) which have been prepared in packaged form for use by the States in their training programs, and by other organizations having a need to present this type of training. Each course package consists of:

1. An instructor's guide containing material related to course planning and conduct.

2. a training manual for use by the participants, containing the course subject matter, and

3. supportive visual and audiovisual training aids used by NTOTC in presenting the course. These are available on loan to those offering the course. The content of each slide is reproduced in this manual.

This guide reflects the manner in which the course has been offered by NTOTC. It is intended to assist the organization offering the training, and should not be considered as an inflexible method of presenting the course. Some may want to follow the format exactly as presented--others may not. In either case, this guide should prove helpful in reducing the amount of originial developmental work required, and in suggesting methods and approaches when modifications of the course plan presented herein are being considered.

Organization of the Guide

This instructor's guide consists of two major parts. Part I contains information required for course planning and management. Part II consists of a series of Instructional Package Worksheets (IPW's) which set forth learning objectives and the instructional approach used by NTOTC for each topic included in the training manual.

Analytical Methodology for Regulatory Programs

It is essential that analytical procedures taught in the course conform to those prescribed for use in USEPA regulatory programs. These procedures are identified in regulations which appear first in the Federal Register, and which are later codified in Title 40 of the annual edition of the Code of Federal Regulations.

For the National Pollutant Discharge Elimination System (NPDES), Part 136 of Title 40, "Guidelines Establishing Test Procedures for the Analysis of Pollutants", specifies methods to be used for the measurement of contaminants in wastewater.

For drinking water, Part 141 of Title 40, "National Interim Primary Drinking Water Regulations" (NIPDWR), specifies methods to be used in determining the level of contaminants in finished water.

The Instructional Package Worksheet on Compliance Monitoring in Part II of this guide provides details concerning EPA regulations for these programs.

For additional information concerning this course, or other packaged courses, contact:

Director, NTOTC
U. S. Environmental Protection Agency
Cincinnati, Ohio 45268

PART I COURSE PLANNING AND MANAGEMENT

This section of the manual is concerned with the administrative aspects of planning, preparing, and conducing the course.

A. COURSE DESCRIPTION

This description was prepared for course presentation at the NTOTC and may be useful to others in course-related publicity.

BACTERIOLOGICAL METHODS IN WATER QUALITY CONTROL PROGRAMS

5 days

This course is for laboratory personnel who can perform basic bacteriological laboratory procedures such as sample inoculations, transfers, weighings and related skills.

After successfully completing the course, the student will have increased knowledge of all aspects of sampling, analysis and data handling for bacteriological samples as required by Federal Register Guidelines for effluent monitoring and other water quality programs.

The training incorporates classroom instruction and activity sessions, student performance of laboratory assignments and follow-up discussions.

Course topics include characteristics, occurrence, and significance of bacterial indicators of pollution; criteria and standards of bacteriological water quality; sampling programs; collection and handling of samples; standard laboratory test procedures for total and fecal coliforms and fecal streptococci, by membrane filter methods and by multiple dilution tube methods; calculating, summarizing and reporting laboratory data; and analytical quality control procedures.

B. PERSONNEL

The remaining sections in Part I of this manual indicate the personnel associated with this training course when it is presented at the National Training and Operational Technology Center (NTOTC). Their course-related activities are listed below.

1. Training Supervisor -
   Has overall responsibility for the NTOTC training program.

2. Training Coordinator -
   Is responsible for all elements involved in planning and conducting a specific course.

3. Course Secretary -
   Performs all course-related clerical duties.

4. Instructor -
   For assigned topics, is responsible for planning instructional approach, developing instructional materials, and delivering the instruction. During laboratory sessions, another instructor may be designated to assist the (primary) instructor so that participants can be provided as much individualized attention as possible.

5. Laboratory Assistant -
   Assists in the prepartion of laboratory reagents; assembles equipment; is available as required during laboratory exercises.

C. SUMMARY PLAN FOR THE COURSE AND COURSE SCHEDULE

A convenient format to use in the early stages of devising a course plan is a day-to-day assignment of time blocks based on estimates by instructors of the training time required for each parameter. (An example is on the next page.) Using available time as a first criterion will allow a variety of possible sequences. One such sequence, which has been successfully used by NTOTC to conduct this course in the past, begins on page 2-3. Examples of other considerations are:

1. If some equipment must be used in more than one test, schedule another topic between the two tests to allow time for the required clean-up.

2. Schedule the topics so each instructor alternates between prime and assistant responsibilities to allow time for preparations which must be done right before training sessions.

3. If one procedure requires skills taught in another procedure, order the presentations accordingly.

SUMMARY PLAN FOR THE COURSE

MONDAY		TUESDAY		WEDNESDAY		THURSDAY		FRIDAY	
Activity	Time Hours	Activity	Time Hours	Activity	Time Hours	Activity	Time Hours	Activity	Time Hours
Registration	1/4	Membrane Filter	3/4	Use of Tables of MPN	3/4	Collection & Handling of Bact. Samples	3/4	Discussion & Review	1/2
Welcome & Course Objectives	1/4	MF Equip. & Its Prep. for Lab Use	3/4	Verified MF Tests	1/2			Quiz	1 1/4
Compliance Meth.	1	Exam. of Water for Colifrom & Fecal Streptococcal Groups (MPN)	1 1/4	Bact. Lab: Equipment, Material, & Supplies	1	Intro to Statistics & Geometric Means	2	Lab Completion	1/2
Chlorine Deter. and Turbidity	1 1/4							Course Closing	1/2
Bacteriological Indicators of WQ	1								
Lunch	1	Lunch	1	Lunch	1	Lunch	1		
Lab Briefing	3/4	Media & Solutions for MPN Methods	1	Colony Counting on MF Filters	1/2	Lab Briefing	3/4		
MPN Procedures (laboratory)	1 1/4	Principles of Cultural Media for use with MF	3/4	Select. of MF Sample Filt. Vol.	1/2	MPN Proc. (lab)	1/4		
MF Media Prep. (laboratory)	1	Lab Briefing	3/4	Lab Briefing	1/2	Verfication (lab)	1/4		
MF Verification (laboratory)	1/2	MPN Proc. (lab)	1/2	MPN Proc. (lab)	1/4	MF Proc. (lab)	2 3/4		
		MF Proc. (lab)	1/2	MF Proc. (lab)	1 3/4				
		MF Ver. (lab)	1/4	MF Verification (lab)	1/4				
TOTAL	8 1/4	TOTAL	7 1/2	TOTAL	7	TOTAL	7 3/4	TOTAL	2 3/4

4. Sample Course Schedule

BACTERIOLOGICAL METHODS IN WATER QUALITY CONTROL PROGRAMS (120.4)

(Location)
(Date)

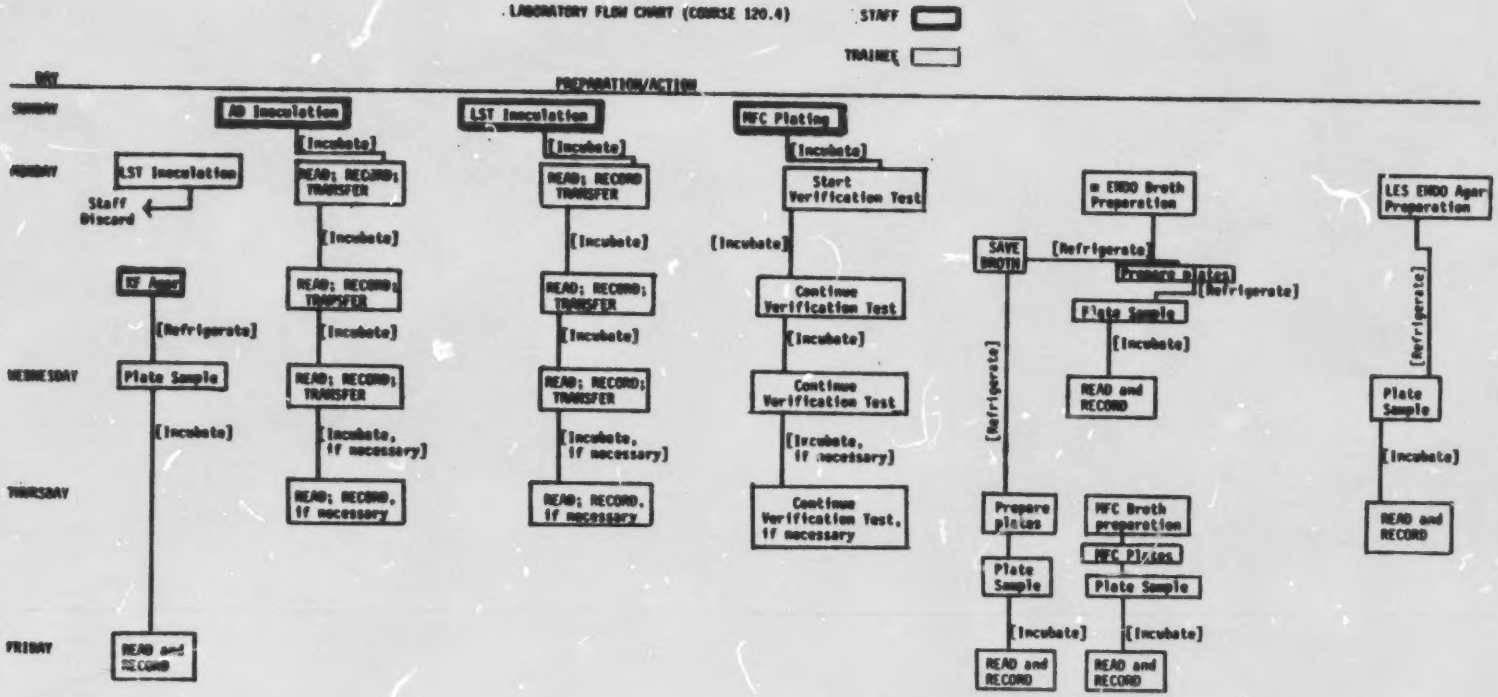
AGENDA

Course Coordinator: Laboratory Assistant:

DAY & TIME	SUBJECT	OUTLINE	INSTRUCTOR*
Monday			
8:30 - 9:00	Welcome, Course Registration and Objectives		Course Coordinator Course Secretary
9:00 - 10:00	Compliance Methodology	1	Bacteriologist #1
10:00 - 11:15	Chlorine Determinations and Turbidity	16	Bacteriologist #2
11:15 - 12:15	Bacteriological Indicators of Water Quality	2	Bacteriologist #1
12:15 - 1:15	Lunch		
1:15 - 4:45	Laboratory Briefing and Laboratory		Staff
Tuesday			
8:30 - 9:15	The Membrane Filter	6	Bacteriologist #1
9:15 - 10:00	MF Equipment and its Preparation for Laboratory Use	7	Bacteriologist #2
10:15 - 10:15	Break		
10:15 - 11:30	Examination of Water for Coliform & Fecal Streptococcal Groups (MPN)	3	Bacteriologist #1
11:30 - 12:30	Lunch		
12:30 - 1:20	Media & Solutions for MPN Methods	4	Bacteriologist #2
1:30 - 2:15	MF - Principles of Culture Media	9	Bacteriologist #1
2:30 - 4:30	Laboratory Briefing and Laboratory		Staff

*For the actual course presentation, insert the name of the primary instructor opposite the title of his/her assignment. Assistant instructors are designated ( ).

DAY & TIME	SUBJECT	OUTLINE	INSTRUCTOR*
Wednesday			
8:30 - 9:15	Use of Tables of MPN	5	Bacteriologist #1
9:25 - 10:05	Verified MF Tests	13	Bacteriologist #2
10:05 - 10:20	Break		
10:20 - 11:30	Bacteriological Laboratory: Equipment, Materials & Supplies		Bacteriologist #1
11:30 - 12:30	Lunch		
12:30 - 1:10	Colony Counting on Membrane Filters	12	Bacteriologist #2
1:20 - 1:50	Selection of MF Sample Filtration Volumes	10	Bacteriologist #1
2:00 - 4:45	Laboratory Briefing & Laboratory		Staff
Thursday			
8:30 - 9:15	Collecting and Handling of Bacteriological Samples	14	Bacteriologist #2
9:25 - 11:20	Introduction to Statistics & Geometrical Means		Bacteriologist #1
11:30 - 12:30	Lunch		
12:30 - 4:30	Laboratory Briefing & Laboratory Studies		Staff
Friday			
8:30 - 9:00	Discussion & Review		Staff
9:25 - 11:20	Quiz & Review		Staff
10:15 - 10:50	Laboratory Exercise Completion		Staff
11:00 - 11:30	Course Closing		Staff


LABORATORY FLOW CHART (COURSE 120.4)
STAFF
TRAINEE
DAY
PREPARATION/ACTION
SUNDAY
MONDAY
TUESDAY
WEDNESDAY
THURSDAY
FRIDAY
AB Inoculation
LST Inoculation
Staff Discard
[Incubate]
READ; RECORD; TRANSFER
[Incubate]
READ; RECORD; TRANSFER
[Incubate]
READ; RECORD; TRANSFER
[Incubate, if necessary]
READ; RECORD, if necessary
EF Agar
[Refrigerate]
Plate Sample
[Incubate]
READ and RECORD
LST Inoculation
[Incubate]
READ; RECORD TRANSFER
[Incubate]
READ; RECORD; TRANSFER
[Incubate]
READ; RECORD; TRANSFER
[Incubate, if necessary]
READ; RECORD, if necessary
MFC Plating
[Incubate]
Start Verification Test
[Incubate]
Continue Verification Test
[Incubate]
Continue Verification Test
[Incubate, if necessary]
Continue Verification Test, if necessary
m ENDO Broth Preparation
[Refrigerate]
SAVE BROTH
[Refrigerate]
Prepare plates
[Refrigerate]
Plate Sample
[Incubate]
READ and RECORD
Prepare plates
[Refrigerate]
Plate Sample
[Incubate]
READ and RECORD
MFC Broth preparation
MFC Plates
Plate Sample
[Incubate]
READ and RECORD
LES ENDO Agar Preparation
[Refrigerate]
Plate Sample
[Incubate]
READ and RECORD

D. MILESTONES IN COURSE PLANNING AND PREPARATION

The following pages list major areas of course responsibilities in a chronological order to facilitate orderly and timely accomplishment. The table also serves as an example for assignment of these responsibilities to various staff members. It has been successfully used by NTOTC to conduct this course in the past.

The table headings are job titles associated with the listed tasks. A suggested staff is cited, including a laboratory assistant. It is recognized, however, that staff is often limited and on individual may serve in several of the defined roles. Having this summary according to an ideal situation should facilitate an equitable division of the required tasks among fewer persons.

Before using the milestones table, decisions must be made about the course content. It may be desirable to teach the approved test profor parameters that are not included in this package, but that are required locally to meet regulatory requirements. In that case, the table must be changed. Delete items identified for topics you omit and the items needed for the topics you want to add.

	Training Supervisor	Course Coordinator	Course Secretary	Instructor #1	Instructor #2	Lab Assistant
5 TO 6 MONTHS BEFORE COURSE						
Determination of the need and decision to have course.	x					
Designation of Course Director and Course Secretary.	x					
Review responsibilities.		x				
Review responsibilities.			x			
Commit classroom and laboratory facilities.	x	x				
Develop and release Course Announcement including location, date, general statement of course content and training objectives.		x	x			
Prepare all forms and information sheets related to student registration procedures.		x	x			
Decide on staff members.	x	x				
4 TO 5 MONTHS BEFORE COURSE						
Receive, review, act upon Course Applications, continuing until course begins.		x	x			
Maintain records on deposition of each application, continuing through course.			x			
Inventory Staff Guides, Order needs.		x	x			

	Training Supervisor	Course Coordinator	Course Secretary	Instructor #1	Instructor #2	Lab Assistant
3 MONTHS BEFORE COURSE						
Commit all staff members who will participate in Course.	x	x	x	x		x
Develop Milestone Checklist for Course.		x	x			
Distribute copies to staff of Milestones, Staff Guide, Student Text and any other pertinent training resources.	x	x	x			
Review responsibilities.			x	x		x
Assign topics to Primary (P) and Assistant (A) Instructors:	x	x				
Compliance Methodology			P			
Chlorine & Turbidity				P		
Indicators of Water Quality			P			
Day One Laboratory			A	P		
Membrane Filter			P			
MF Equipment				P		
MPN Examinations			P			
MPN Media				P		
MF Media			P			
Day Two Laboratory			P	A		
MPN Tables			P			
Verified Tests				P		
Laboratory Equipment			P			
MF Colony Counting				P		
MF Sample Volumes			P			
Day Three Laboratory			A	P		
Bacteriological Samples				P		
Statistics			P			
Day Four Laboratory			A	P		
Post Course Quiz			P	A		
Day Five Laboratory			A	P		
Develop summary plan for course		x	x	x	x	x
Inventory laboratory equipment/ supplies. List and commit lending sources. Order rest.		x	x	x	x	x
Inventroy classroom equipment/ supplies. List and commit lending sources. Order rest. (Continued)		x	x			

	Training Supervisor	Course Coordinator	Course Secretary	Instructor #1	Instructor #2	Lab Assistant
Inventory student reference texts. Order needs.		x	x			
2 MONTHS BEFORE COURSE						
Finalize Course Schedule (Agenda).		x	x	x	x	x
Request laboratory/classroom needs from lending sources.		x	x	x	x	x
Request training aids from lending sources.		x	x	x	x	
6 WEEKS BEFORE COURSE						
Check out operation of all major pieces of equipment.				x	x	x
Primary and Assistant Instructors go through laboratory procedures in student reference texts, using IPWs to standardize instructions for students.				x	x	
1 MONTH BEFORE COURSE						
Summary (to date) to staff of registered students, continuing to course beginning.		x	x			
Check on progress of staff preparations for instruction, continuing through course.		x		x	x	
Prepare all administrative forms and materials needed for course presentation.		x	x			
Plan and rehearse classroom presentations using all required training aids. Finalize. .				x	x	

(Continued)

	Training Supervisor	Course Coordinator	Course Secretary	Instructor #1	Instructor #2	Lab Assistant
Obtain any duplicated instructional materials (data sheets, etc.).			x	x	x		x
Review summary of laboratory equipment and supply needs for expected number of students doing the selected procedures:		x					x
Clean all glassware and equipment required by students.							x
Assemble other student equipment and supplies.							x
2 WEEKS BEFORE COURSE							
Arrange for security of classroom and laboratory.		x	x				
Make reagents, media, solutions, etc required by students.				x	x		x
Determine approximate range of parameters in samples for course.				x	x		
Arrange for disposal of contaminated wastes.				x	x		x

(Continued)

	Training Supervisor	Course Coordinator	Course Secretary	Instructor #1	Instructor #2	Lab Assistant
Give Laboratory Assistant final list of equipment and supplies to be at each laboratory position. Discuss arrangement of shared equipment.				x	x	
1 WEEK BEFORE COURSE						
Inform building food service of number of expected students and course lunch times (as appropriate).			x			
3 DAYS BEFORE COURSE						
Finalize seating arrangement for classroom.		x				
Assemble course materials in classroom (student texts, administrative materials, etc.). Distribute as appropriate.		x	x			
Ready classroom instructional aids (boards, erasers, etc.)		x	x			x
Check out all classroom equipment (electrical systems, PA, projection equipment) and obtain back-up accessories (bulbs, etc.).		x				
COURSE OPENING						
Conduct opening exercises. Participate in course opening.	x	x	x	x	x	x
Complete any required student records, including roster.		x	x			

(Continued)

	Training Supervisor	Course Coordinator	Course Secretary	Instructor #1	Instructor #2	Lab Assistant
Prepare course certificates if needed at end of week.		x	x			
EVERY DAY OF COURSE						
Maintain general supervision of course.		x				
Prepare materials and/or samples on day of test.			x	x		x
Obtain samples for each test on day of test.			x	x		x
When assistant instructor, make ary student evaluation records requested by the lead instructor.			x	x		
When primary instructor, compile evaluation record for each student, if required.			x	x		
Keep any general records (e.g. attendance) as required to document successful course completion.		x	x	x		

3-7

	Training Supervisor	Course Coordinator	Course Secretary	Instructor #1	Instructor #2	Lab Assistant
SECOND-LAST DAY OF COURSE						
Distribute course critique sheet to students.		x				
LAST DAY OF COURSE						
Check and sign course certificates if applicable		x				
Collect students' critique sheets.		x				
Conduct closing exercises.		x				
Participate in course closing.	x		x	x	x	x
Clean up classroom and laboratory.		x	x	x	x	x
WITHIN TWO WEEKS OF COURSE PRESENTATION						
Return or replace any borrowed classroom equipment/ supplies.		x	x			
Return or replace any borrowed laboratory equipment/ supplies.				x	x	x
Return or replace any borrowed training aids.		x	x	x	x	
Order repairs or replacements of own equipment used in course.		x	x	x	x	
Complete and file evaluation records on all students in predetermined area.			x	x	x	
See that all students have been appropriately notified of their degree of success in completing the course.		x	x			

	Training Supervisor	Course Coordinator	Course Secretary	Instructor #1	Instructor #2		Lab Assistant
Participate in staff session on evaluation of course and recommendations for future offerings.	x	x	x	x	x		x
Prepare course summary/ evaluation report.		x					
Complete and file entire course records in mutually determined area.		x	x				

E. INSTRUCTIONAL RESOURCES

Most training institutions will make the fullest possible use of available instructional resources. The purpose of this section is to describe the nature, sources and availability of instructional resources suggested for use with this course.

1. Student reference text and Instructor's Guide for the course, "Bacteriological Methods in Water Quality Control Programs." Ordering information may be obtained from:

   U. S. Environmental Protection Agency
   National Training and Operational Technology Center
   Cincinnati, Ohio 45268
   (513) 684-7501

2. Slides and Slide/Tape Units (See IPW Sections VIII B and XI for a description).

   a) What is available, according to topic:

      (1) Chlorine Determinations and Turbidity

         (a) X-21, chlorine, 36 slides

         (b) X-30, Turbidity, 10 slides

      (2) Bacteriological Indicators

         (a) Bacteriological Indicators, 53 slides.

      (3) Membrane Filter/Equipment/procedures

         (a) X-41 Membrane Filter, 12 slides

         (b) X-33 MF Colony Counting, 17 slides

      (4) MPN

         (a) Examination of water for Coliform and Fecal Streptococcal Groups (MPN), 40 slides

      (5) Collection and Handling and Samples

         (a) Collection and Handling of Bacteriological Samples, 14 slides

      (6) Statistics

         (a) XT-86 Geometric Means (Parts I, II, III) time of three tpaes is 35 minutes, with 78 slides, 3 scripts, and a handout.

All units described in 2. above are available on scheduled loan from NTOTC to institutions conducting this course. Requests should contain the information items on the "Request for Loan" form at the end of this section. Send requests to the National Training and Operational Technology Center at the address on page 4-1.

It is urged that materials desired from NTOTC for a specific course offering be requested in a single, consolidated communication. This will give greatest assurance of a well-coordinated response. Because these requests ordinarily will cover a number of different items, telephonic requests should not be made.

Requests should be timely. To assure effective delivery in time for use in the course, requests should be received at NTOTC at least 45 days prior to the course date. NTOTC will, in turn, make every effort to assure that the requested materials are delivered to the requesting institution several days prior to the start of the course in which they are to be used. This will permit review and practice by the instructional staff for the most effective use of such resources.

It is expected that all borrowed resources be returned to NTOTC within two weeks after completion of the course in which they are used.

With returned borrowed training resources, it is requested that the user provide NTOTC with an evaluation of the training resourse(s) used. In this manner, the experience of users can be a factor in continuous improvements and responses to problems in using the resources. All reports on the use of such resources should include the number of students with whom the materila was used.

3. Supportive References:

   a) "Standard Methods for the Examination of Water and Wastewater" (14th edition), APHA, AWWA, WPCF. Available from Publication Office, American Public Health Association, Inc., 1015 18th Street, N.W., Washington, DC 20036.

4. Instructional Resources Already in Possession of the Training Institution

   a) Many training organizations prefer to develop, or have developed, their own texts and audiovisual training resources.

REQUEST FOR LOAN
AUDIOVISUAL INSTRUCTIONAL UNIT

Title and Catalog No. _____

_____

Intended Use: _____

_____

Preferred Date of Use: _____

Alternate Date: _____

BORROWER'S NAME _____

Title _____

Organization _____

Address _____

_____
(Zip)

Phone Number (include
Area Code): _____

To prevent duplication when choosing Audiovisual Materials, please note in the Topical Index that some units are carried in more than one mode.

There is no charge for use of the Audiovisual Instructional Units. However, the *BORROWER* assumes financial responsibility for the value of all loaned instructional materials.

Unless special arrangements are made with the loaning office, units should be returned within two weeks. Return the unit by *REGISTERED, CERTIFIED* or *INSURED MAIL IMMEDIATELY* after use.

FILMS MUST BE RETURNED IN 3 DAYS.

EPA-171 (Cin) (Rev. 7-78) 4-3

F. LABORATORY EQUIPMENT AND SUPPLY REQUIREMENTS

The consolidated list in this section is for overall planning purposes. It was compiled from the Instructional Package Worksheet (IPW) sections (part II of this manual), and IX, "IPW Equipment and Supply Requirements" "Section IX can also be used on a day-to-day basis during the course to prepare for the laboratory exercises.

If an Instructor chooses a different assignment for a topic, some quantities must be changed accordingly. As noted, numbers represent minimum quantities. It is strongly recommended that Instructors provide surplus equipment and additional supplies ready for use in case of need. Many Instructors plan for a margin of at least 10% of extra supplies to provide for student errors, planning miscalculations, or other unforeseen events.

Before using the list, decisions must be made about the course content. It may be desirable to teach the approved test procedures for parameters that are not included in this package, but that are required locally to meet regulatory requirements. In that case, the list must be changed. Delete items identified for topics you omit and add the items needed for the topics you omit and add the items needed for the topics you want to add.

This list can be of great value in pre-course planning, to determine the availability of needed equipment and supplies, and to take action to provide needed resources. Further, this list can be of vital importance when planning for courses to be conducted in field locations. Copies of the list in the hands of the Course Coordinator and a representative of the host organization can be used to determine which will provide needed resources on an item-by-item basis. When the responsibility is assigned/accepted, this can be annotated in the "remarks" column on the copy in the hands of the Course Coordinator and the copy of the representative of the host organization. Each can then use the annotated equipment and supply list as a checklist for carrying out his own agreed - upon responsibilities in preparing for the course.

BACTERIOLOGICAL METHODS IN WATER QUALITY CONTROL PROGRAMS

MATERIAL & EQUIPMENT REQUIREMENTS

Note: Below are shown the materials and equipment required PER STUDENT or laboratory position. Multiply the number by the number of students to obtain required supply.

Note: The symbol "x" means that supply/equipment is available - such as "matches" or "incubator"

NOMENCLATURE	SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
*AD Broth 2X Strength	5					
*AD Broth 1X Strength	20					
*LTS 2X Strength	5					
*LTS 1X Strength	20	27				
BGLB Broth		15	7			
EVA Broth		12	3			
m-ENDO Broth bottle 100ml dehydrated medium		1				
LES ENDO Agar Vial 30ml dehydrated medium		1				
EC Broth		15	9			
Blank dilution 99ml Sterile	2	2	1	1	1	
Rinse Water Sterile (sufficient amount if in flasks - or the following amounts when 99ml blanks are used)			6	4	18	
Sticks, Inoculation Sterile		12	3			
Loop, Inoculation		1	1	1	1	
Dishes, Petri MF Sterile		15		5	12	
Burner gas	x	1	1	1	1	
Needle bacteriological		1				

BACTERIOLOGICAL METHODS IN WATER QUALITY CONTROL PROGRAMS

MATERIAL & EQUIPMENT REQUIREMENTS

NOMENCLATURE	SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
MFC medium prepared plates	1					
MFC medium dehydrated 30ml vial					1	
Rosolic acid prepared in 0.2N NaOH					x	
Incubator, 35 C	x	x	x	x	x	x
Incubator, 44.5 C	x	x	x	x	x	x
Pipet Sterile 10ml	1	3	3	7	5	
Pipet Sterile 1ml	3	4	3	7	3	
Forceps MF w/methanol vial	1		1	1	1	
Marker Wax	1	1	1	1	1	
KF Agar plates prepared				6		
Filter Apparatus MF	1		1	1	1	
Matches	x	x	x	x	x	
Membrane Filters	1		7	12	10	
Discard trays and racks	x	x	x	x	x	
Aprons protective		x (use for week)				
Stereoscope 10-15X fluorescent lighting			x	x	x	x

MATERIAL & EQUIPMENT REQUIREMENTS

NOMENCLATURE	SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
Sample Bottles 250ml size, Sterile, Thiosulfate not required	1	1	1	3	3	
Bottle Sterile 2-4 liters	1	1	1	1	1	
Cylinder 25ml sterile					1	
Cylinder 50ml sterile					1	
Cylinder 100ml sterile					1	

*Note: Inoculate one rack with selected sample - this will be used as instructor test to demonstrate daily reading, recording and procedural sequence.

PART II - INSTRUCTIONAL PACKAGE WORKSHEETS

The Worksheets are for guidance to the Instructor who develops the subject matter covered in the course. These Worksheets are not scripts. The Instructor will need to make extensive and detailed preparation in order to perform the assigned tasks effectively and efficiently. The Instructional Packages do provide a perspective on the background of each analytical procedure, lesson-by-lesson learning achievement levels the students should attain, an indication of available audiovisual and other instructional resources, and an example course of action in pre-course preparation and classroom/laboratory instruction. The Instructor is free to modify the Worksheets to meet individual needs. It should be noted however, that associated additions and deletions will then be required in Sections V through XI of the Worksheet, and in Sections E and F of Part I.

Application of these Instructional Packages will help the Instructor to reduce the time required for planning and organizing a strategy of preparation and instruction. However, time and effort are required for physical preparations for classroom and laboratory instruction; time and effort are required for rehearsals of Instructor performance in classroom and laboratory. These requirements never can be met by such an Instructor's Guide as this; ultimately the Instructor is the key person in assuring that the student acquires the needed knowledge and skills.

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Compliance Methodology

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 60 minutes

IV. **JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE:** Knowledge of this material allows the participant to be aware of regulatory requirements pertaining to the National Pollutant Discharge Elimination System (NPDES) and National Interim Primary Drinking Water Regulations (NIPDWR).

V. **ENTRY LEVEL BEHAVIOR:**

A. None required.

VI. **INSTRUCTIONAL OBJECTIVE:**

A. Terminal Behavior: The participant will be able to use federal registers and pertinent EPA explanatory memos pertaining to approved analytical methodology for compliance with the National Pollutant Discharge Elimination System (NPDES) National Interim Primary Drinking Water Regulations (NIPDWR).

B. Conditions: He/she will be given the training manual, copies of the information (see VII A), and a 60 minute explanation of their applicability and format.

C. Accepted Performance: In attendance to the lecture material covering subject material.

VII. **INSTRUCTIONAL RESOURCES:**

A. Available Media:

1. One outline in the training manual:

a. Federal Register Guidelines Establishing Test Procedures for the Analysis of Pollutants.

2. Handouts:

a. National Pollutant Discharge Elimination System - NPDES:

1) Guidelines Establishing Test Procedures, 40 CFR 136, Federal Register, 12/1/76, pp. 52780 - 52786. A copy is at the end of this IPW, and also an errata sheet. Changes were proposed 12/18/79, pp. 75028-75052 and should be finalized after January, 1981. Check with your EPA Regional Quality Assurance Coordinator to see if the finalized Guidelines have been published. (Name and number is available from EMSL - C 513-684-7301). The Coordinator can supply copies to you.

2) Summary of protocols to apply for approval of an alternative test procedure for regulatory purposes. A copy is at the end of this IPWO.

3) EPA - EMSL memo on "Use of Chemical Test Kits for Compliance Monitoring". A copy is at the end of this IPW.

4) EPA - EMSL memo on "Use of "Prepared" Reagents in NPDES Compliance." A copy is at the end of this IPW.

b. National Interim Primary Drinking Water Regulations _ NIPDWR:

1) NIPDWR, 40 CFR 141, Federal Register, 12/24/75, pp 59566-59574. A copy is at the end of this IPW.

2) Amendments to NIPDWR were proposed 7/19/79, 40 CFR Part 141, Federal Register, 8/27/80, pp 57332 - 57346. Copies of pp. 57343 - 57346 are at the end of this IPW. The pages contain the current (8/80), approved analytical requirements for drinking water analysis. These include an update of the approved methods presented in the 12/24/75 NIPDWR and also alternate analytical techniques approved by EPA since that date. Check with your EPA Regional Quality Assurance Coordinate to get copies of any additional, pertinent Federal Register notices published after 8/80.

B. Suggested Media:

OPTIONAL: Copies of the methods manuals cited for NPDES and NIPDWR could be available for display/inspection.

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Contact your EPA Regional Quality Assurance Coordinator to check if any federal registers pertaining to NPDES or to NIPDWR have been issued since the ones referenced in VII A above (8/80). If so, obtain copies from the Coordinator for your class.

2. Thoroughly review all pertinent federal register information and handout materials. Since the regulations are legally binding, it is imperative to import correct and current information to the participants.

3. Prepare the lesson using the sequencing below or your own organization of the materials.

4. Duplicate copies of all the handout materials. Most can be copied back-to-back, but be careful to keep each handout as an entity so participants can use each independent of the other. Collate the handouts into a packet for each participant to minimize distribution time.

B. Sequencing:

1. Hand out the packets of information.

2. Introduction

   a. Choice of methodology affects validity and reliability of data.

   b. Analytical programs affected by federal legislation include those involving point source discharges (NPDES), and drinking water (NIPDWR).

3. National Pollutant Discharge Elimination System (NPDES). CAUTION: Use the current federal register. The NPDES Guidelines are scheduled for update after January, 1981.

   a. Applicability of the regulations. See 12/1/76, p. 52780, paragraph 2.

   b. Overview of Table 1

      1) Column 1: Alphabetical listing of parameters and units. Note subcategories.

      2) Column 2: method listings and possibly specification of a pre-treatment and/or method choice, e.g. Acidity end point is to be pH of 8.2.

      3) Remaining columns list sources (with page numbers) of approved methods. You can display copies of the cited manuals at this time and leave them to be available for inspecting by participants.

      4) Note the location (by number) of the parameters to be taught during the course. Also note the method to be used for course laboratory sessions.

      5) Note any errata (handout).

   c. Go over highlights of applying for approval of Alternative Test Procedures (handout)

      1) Limited use

      2) Nationwide use

   d. Go over highlights of the memo on "Use of Chemical Test Kits for Compliance Monitoring "(handout).

   e. Note highlights of the memo on "Use of "Prepared" Reagents in NPDES Compliance" (handout).

4. Drinking Water Regulations. CAUTION: Use current federal registers. The 12/24/75 federal register is the current (8/80) National Interim Primary Drinking Water Regulation (NIPDWR), along with the Amendments to NIPDWR which were finalized 8/27/80.

   a. Applicability of the regulations. See 12/24/75, p. 59566, "Water Systems Covered", and p 59570, "Subpart A - General."

   b. Maximum contaminant levels and the discussion of sampling and analytical requirments begin on page 59571.

c. The analytical methodology in the 12/24/75 regulation was updated and expanded in the 8/27/80 regulation. Therefore, use pp 57343 - 57346 from the 8/27/80 Federal Register to cite the location of the listing of the current approved methods: 141.21 Microbiological ... Requirements. (There was no change in the reference for the source of chlorine methodology to be used when chlorine risidual monitoring is substituted for microbiological testing. The reference in the 12/24/75 NIPDWR, p. 59572, h), is the current (8/80) reference). In 8/27/80, also highlight section 141.28, approved Laboratories and section 141.27, Alternate Analytical Techniques.

5. A 10 minute discussion period should be scheduled later to answer any questions.

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. <u>For each student</u>:

1. Copies of handout materials

X. IPW REAGENT REQUIREMENTS:

A. None

federal register

WEDNESDAY, DECEMBER 1, 1976

PART II:

ENVIRONMENTAL PROTECTION AGENCY

■

WATER PROGRAMS

Guidelines Establishing Test Procedures for the Analysis of Pollutants

Amendments

Title 40—Protection of Environment

CHAPTER I—ENVIRONMENTAL PROTECTION AGENCY

SUBCHAPTER D—WATER PROGRAMS

[FRL 630-4]

PART 136—GUIDELINES ESTABLISHING TEST PROCEDURES FOR THE ANALYSIS OF POLLUTANTS

Amendment of Regulations

On June 9, 1975, proposed amendments to the Guidelines Establishing Test Procedures for the Analysis of Pollutants (40 CFR 136) were published in the FEDERAL REGISTER (40 FR 24535) as required by section 304(g) of the Federal Water Pollution Control Act Amendments of 1972 (86 Stat. 816, et seq., Pub. L. 92-500, 1972) hereinafter referred to as the Act.

Section 304(g) of the Act requires that the Administrator shall promulgate guidelines establishing test procedures for the analysis of pollutants that shall include factors which must be provided in: (1) any certification pursuant to section 401 of the Act, or (2) any permit application pursuant to section 402 of the Act. Such test procedures are to be used by permit applicants to demonstrate that effluent discharges meet applicable pollutant discharge limitations and by the States and other enforcement activities in routine or random monitoring of effluents to verify compliance with pollution control measures.

Interested persons were requested to submit written comments, suggestions, or objections to the proposed amendments by September 7, 1975. One hundred and thirty-five letters were received from commenters. The following categories of organizations were represented by the commenters: Federal agencies accounted for twenty-four responses; State agencies accounted for twenty-six responses; local agencies accounted for seventeen responses; regulated major dischargers accounted for forty-seven responses; trade and professional organizations accounted for eight responses; analytical instrument manufacturers and vendors accounted for seven responses; and analytical service laboratories accounted for six responses.

All comments were carefully evaluated by a technical review committee. Based upon the review of comments, the following principal changes to the proposed amendments were made:

(A) *Definitions.* Section 136.2 has been amended to update references: Twenty commenters, representing the entire spectrum of responding groups pointed out that the references cited in §§ 136.2 (f), 136.2(g), and 136.2(h) were out-of-date; §§ 136.2(f), 136.2(g), and 136.2(h), respectively, have been amended to show the following editions of the standard references: "14th Edition of Standard Methods for the Examination of Water and Waste Water;" "1974 EPA Manual of Methods for the Analysis of Water and Waste;" and "Part 31, 1975 Annual Book of ASTM Standards."

(B) *Identification of Test Procedures.* Both the content and format of § 136.3, "Table I, List of Approved Test Procedures" have been revised in response to twenty-one comments received from State and local governments, major regulated dischargers, professional and trade associations, and analytical laboratories.

Table I has been revised by:

(1) The addition of a fourth column of references which includes procedures of the United States Geological Survey which are equivalent to previously approved methods.

(2) The addition of a fifth column of miscellaneous references to procedures which are equivalent to previously approved methods.

(3) Listing generically related parameters alphabetically within four subcategories: bacteria, metals, radiological and residue, and by listing these subcategory headings in alphabetic sequence relative to the remaining parameters.

(4) Deleting the parameter "Algicides" and by entering the single relevant algicide, "Pentachlorophenol" by its chemical name.

(C) *Clarification of Test Parameters.* The conditions for analysis of several parameters have been more specifically defined as a result of comments received by the Agency:

(1) In response to five commenters representing State or local governments, major dischargers, or analytical instrument manufacturers, the end-point for the alkalinity determination is specifically designated as pH 4.5.

(2) Manual digestion and distillation are still required as necessary preliminary steps for the Kjeldahl nitrogen procedure. Analysis after such distillation may be by Nessler color comparison, titration, electrode, or automated phenolate procedures.

(3) In response to eight commenters representative of Federal and State governments, major dischargers, and analytical instrument manufacturers, manual distillation at pH 9.5 is now specified for ammonia measurement.

(D) *New Parameters and Analytical Procedures.* Forty-four new parameters have been added to Table I. In addition to the designation of analytical procedures for these new parameters, the following modifications have been made in analytical procedures designated in response to comments.

(1) The ortho-tolidine procedure was not approved for the measurement of residual chlorine because of its poor accuracy and precision. Its approval had been requested by seven commenters representing major dischargers, State, or local governments, and analytical instrument manufacturers. Instead, the N,N-diethyl-p-phenylenediamine (DPD) method is approved as an interim procedure pending more intensive laboratory testing. It has many of the advantages of the ortho-tolidine procedure such as low cost, ease of operation, and also is of acceptable precision and accuracy.

(2) The Environmental Protection Agency concurred with the American Dye Manufacturers' request to approve its procedure for measurement of color, and copies of the procedure are now available at the Environmental Monitoring and Support Laboratory, Cincinnati (EMSL-CI).

(3) In response to three requests from Federal, State governments, and dischargers, "hardness," may be measured as the sum of calcium and magnesium analyzed by atomic absorption and expressed as their carbonates.

(4) The proposal to limit measurement of fecal coliform bacteria in the presence of chlorine to only the "Most Probable Number" (MPN) procedure has been withdrawn in response to requests from forty-five commenters including State pollution control agencies, permit holders, analysts, treatment plant operators, and a manufacturer of analytical supplies. The membrane filter (MF) procedure will continue to be an approved technique for the routine measurement of fecal coliform in the presence of chlorine. However, the MPN procedure must be used to resolve controversial situations. The technique selected by the analyst must be reported with the data.

(5) A total of fifteen objections, representing the entire spectrum of commenters, addressed the drying temperatures used for measurement of residues. The use of different temperatures in drying of total residue, dissolved residue and suspended residue was cited as not allowing direct intercomparability between these measurements. Because the intent of designating the three separate residue parameters is to measure separate waste characteristics (low drying temperatures to measure volatile substances, high drying temperatures to measure anhydrous inorganic substances), the difference in drying temperatures for these residue parameters must be preserved.

(E) *Deletion of Measurement Techniques.* Some measurement techniques that had been proposed have been deleted in response to objections raised during the public comment period.

(1) The proposed infrared spectrophotometric analysis for oil and grease has been withdrawn. Eleven commenters representing Federal or State agencies and major dischargers claimed that this parameter is defined by the measurement procedure. Any alteration in the procedure would change the definition of the parameter. The Environmental Protection Agency agreed.

(2) The proposed separate parameter for sulfide at concentrations below 1 mg/l, has been withdrawn. Methylene blue spectrophotometry is now included in Table I as an approved procedure for sulfide analysis. The titrimetric iodine procedure for sulfide analysis may only be used for analysis of sulfide at concentrations in excess of one milligram per liter.

(F) *Sample Preservation and Holding Times.* Criteria for sample preservation and sample holding times were requested by several commenters. The reference for sample preservation and holding time criteria applicable to the Table I parameters is given in footnote (1) of Table I.

(G) *Alternate Test Procedures.* Comments pertaining to § 136.4, Application for Alternate Test Procedures, included objections to various obstacles within

these procedures for expeditious approval of alternate test procedures. Four analytical instrument manufacturers commented that by limiting of application for review and/or approval of alternate test procedures to NPDES permit holders, § 136.4 became an impediment to the commercial development of new or improved measurement devices based on new measurement principles. Applications for such review and/or approval will now be accepted from any person. The intent of the alternate test procedure is to allow the use of measurement systems which are known to be equivalent to the approved test procedures in waste water discharges.

Applications for approval of alternate test procedures applicable to specific discharges will continue to be made only by NPDES permit holders, and approval of such applications will be made on a case-by-case basis by the Regional Administrator in whose Region the discharge is made.

Applications for approval of alternate test procedures which are intended for nationwide use can now be submitted by any person directly to the Director of the Environmental Monitoring and Support Laboratory in Cincinnati. Such applications should include a complete methods write-up, any literature references, comparability data between the proposed alternate test procedure and those already approved by the Administrator. The application should include precision and accuracy data of the proposed alternate test procedure and data confirming the general applicability of the test procedure to the industrial categories of waste water for which it is intended. The Director of the Environmental Monitoring and Support Laboratory, after review of submitted information, will recommend approval or rejection of the application to the Administrator, or he will return the application to the applicant for more information. Approval or rejection of applications for test procedures intended for nationwide use will be made by the Administrator, after considering the recommendation made by the Director of the Environmental Monitoring and Support Laboratory, Cincinnati. Since the Agency considers these procedures for approval of alternate test procedures for nationwide use to be interim procedures, we will welcome suggestions for criteria for approval of alternate test procedures for nationwide use. Interested persons should submit their written comments in triplicate on or before June 1, 1977 to: Dr. Robert B. Medz, Environmental Protection Technologist, Monitoring Quality Assurance Standardization, Office of Monitoring and Technical Support (RD-680), Environmental Protection Agency, Washington, D.C. 20460.

(H) *Freedom of Information.* A copy of all public comments, an analysis by parameter of those comments, and documents providing further information on the rationale for the changes made in the final regulation are available for inspection and copying at the Environmental Protection Agency Public Information Reference Unit, Room 2922, Waterside Mall, 401 M Street, SW., Washington, D.C. 20460, during normal business hours. The EPA information regulation 40 CFR 2 provides that a reasonable fee may be charged for copying such documents.

Effective date: These amendments become effective on April 1, 1977.

Dated: November 19, 1976.

JOHN QUARLES,
Acting Administrator,
Environmental Protection Agency.

Chapter I, Subchapter D, of Title 40, Code of Federal Regulations is amended as follows:

1. In § 136.2, paragraphs (f), (g), and (h) are amended to read as follows:

§ 136.2 Definitions.

• • • • •

(f) "Standard Methods" means *Standard Methods for the Examination of Water and Waste Water*, 14th Edition, 1976. This publication is available from the American Public Health Association, 1015 18th Street, N.W., Washington, D.C. 20036.

(g) "ASTM" means *Annual Book of Standards, Part 31, Water, 1975.* This publication is available from the American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pennsylvania 19103.

(h) "EPA Methods" means *Methods for Chemical Analysis of Water and Waste, 1974.* Methods Development and Quality Assurance Research Laboratory, National Environmental Research Center, Cincinnati, Ohio 45268; U.S. Environmental Protection Agency, Office of Technology Transfer, Industrial Environmental Research Laboratory, Cincinnati, Ohio 45268. This publication is available from the Office of Technology Transfer.

2. In § 136.3, the second sentence of paragraph (b) is amended, and a new paragraph (c) is added to read as follows:

§ 136.3 Identification of test procedures.

• • • • •

(b) • • • Under such circumstances, additional test procedures for analysis of pollutants may be specified by the Regional Administrator or the Director upon the recommendation of the Director of the Environmental Monitoring and Support Laboratory, Cincinnati.

(c) Under certain circumstances, the Administrator may approve, upon recommendation by the Director, Environmental Monitoring and Support Laboratory, Cincinnati, additional alternate test procedures for nationwide use.

3. Table I of § 136.3 is revised by listing the parameters alphabetically; by adding 44 new parameters; by adding a fourth column under references listing equivalent United States Geological Survey methods; by adding a fifth column under references listing miscellaneous equivalent methods; by deleting footnotes 1 through 7 and adding 24 new footnotes, to read as follows:

• • • • •

TABLE I.—*List of approved test procedures* [1]

Parameter and units	Method	1974 EPA methods	14th ed. standard methods	References (page nos.) Pt. 31 1975 ASTM	References (page nos.) USGS methods [2]	Other approved methods
1. Acidity, as $CaCO_3$ [3], milligrams per liter.	Electrometric end point (pH of 8.2) or phenolphthalein end point.	1	273(4d)	116	40	[4](607)
2. Alkalinity, as $CaCO_3$ [3], milligrams per liter.	Electrometric titration (only to pH 4.5) manual or automated, or equivalent automated methods.	3 5	278	111	41	[4](607)
3. Ammonia (as N), milligrams per liter.	Manual distillation [5] (at pH 9.5) followed by nesslerization, titration, electrode, Automated phenolate.	159 165 168	410 413 416	237	116	[4](614)
BACTERIA						
4. Coliform (fecal) [3], number per 100 ml.	MPN; [6] membrane filter.		922 927		[7](45)	
5. Coliform (fecal) [3] in presence of chlorine, number per 100 ml.	do. [6 8]		922 928, 927			
6. Coliform (total), [3] number per 100 ml.	do. [6]		916 928		[7](38)	
7. Coliform (total) [3] in presence of chlorine, number per 100 ml.	MPN; [6] membrane filter with enrichment.		916 923			
8. Fecal streptococci, [3] number per 100 ml.	MPN; [6] membrane filter, plate count.		943 944 947		[7](50)	
9. Benzidine, milligrams per liter.	Oxidation—colorimetric [9]					
10. Biochemical oxygen demand, 5-d (BOD_5), milligrams per liter.	Winkler (Azide modification) or electrode method.		543		[7](50)	[10](17)
11. Bromide, milligrams per liter.	Titrimetric, iodine-iodate	14		223	56	
12. Chemical oxygen demand (COD), milligrams per liter.	Dichromate reflux	20	550	473	134	[4](610) [10](17)
13. Chloride, milligrams per liter.	Silver nitrate; mercuric nitrate; or automated colorimetric-ferricyanide.	28 31	508 509 513	267 265	[11](46)	[4](615)

See footnotes at end of table.

Parameter and units	Method	1974 EPA methods	14th ed. standard methods	References (page nos.) Pt. 31 1975 ASTM	References (page nos.) USGS methods [1]	Other approved methods
14. Chlorinated organic compounds (except pesticides), milligrams per liter.	Gas chromatography [12]					
15. Chlorine—total residual, milligrams per liter.	Iodometric titration, amperometric or starch-iodine end-point; DPD colorimetric or Titrimetric methods (these last 2 are interim methods pending laboratory testing).	35	318 322 322 320	278		
16. Color, platinum cobalt units or dominant wave length, hue, luminance, purity.	Colorimetric: spectrophotometric or ADMI procedure.[13]	28 30	64 66		82	
17. Cyanide, total,[14] milligrams per liter.	Distillation followed by silver nitrate titration or pyridine pyrazolone (or barbituric acid) colorimetric.	40	361	503	85	[2](322)
18. Cyanide amenable to chlorination, milligrams per liter.	do	40	376	505		
19. Dissolved oxygen, milligrams per liter.	Winkler (Azide modification) or electrode method.	51 56	443 450	368	126	[4](600)
20. Fluoride, milligrams per liter.	Distillation [4] followed by ion electrode; SPADNS; or automated complexone.	65 59 61	390 391 395 614	397 395	93	
21. Hardness—Total, as $CaCO_3$, milligrams per liter.	EDTA titration; automated colorimetric; or atomic absorption (sum of Ca and Mg as their respective carbonates).	68 70	232	161	94	[4](617)
22. Hydrogen ion (pH), pH units.	Electrometric measurement.	230	460	178	120	[4](606)
23. Kjeldahl nitrogen (as N), milligrams per liter.	Digestion and distillation followed by nesslerization, titration, or electrode; automated digestion automated phenolate.	175 145 182	437		122	[4](613)
METALS						
24. Aluminum—Total, milligrams per liter.	Digestion [15] followed by atomic absorption [16] or by colorimetric (Eriochrome Cyanine R).	92	152 171		[11] (19)	
25. Aluminum—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced methods for total aluminum.					
26. Antimony—Total, milligrams per liter.	Digestion [15] followed by atomic absorption.[16]	94				
27. Antimony—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total antimony.					
28. Arsenic—Total, milligrams per liter.	Digestion followed by silver diethyldithiocarbamate; or atomic absorption.[16 18]	8 95	295 288 139		[11] (34) [11] (37)	
29. Arsenic—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total arsenic.					
30. Barium—Total, milligrams per liter.	Digestion [15] followed by atomic absorption.[16]	97	152		82	
31. Barium—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total barium.					
32. Beryllium—Total, milligrams per liter.	Digestion [15] followed by atomic absorption [16] or by colorimetric (Aluminon).	90	122 177		88	
33. Beryllium—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total beryllium.					
34. Boron—Total, milligrams per liter.	Colorimetric (Curcumin)	13	267			
35. Boron—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total boron.					
36. Cadmium—Total, milligrams per liter.	Digestion [15] followed by atomic absorption [16] or by colorimetric (Dithizone).	101	146 152	345	80 [4](619) [19](37)	
37. Cadmium—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total cadmium.					
38. Calcium—Total, milligrams per liter.	Digestion [15] followed by atomic absorption; or EDTA titration.	102	148 150	345	66	
39. Calcium—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total calcium.					
40. Chromium VI, milligrams per liter.	Extraction and atomic absorption; colorimetric (Diphenylcarbazide).	89, 105	192		[4]75 75	
41. Chromium VI—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for chromium VI.					
42. Chromium—Total, milligrams per liter.	Digestion [15] followed by atomic absorption [16] or by colorimetric (Diphenylcarbazide).	105	148 152	345 350	73 77	[4](620)
43. Chromium—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total chromium.					

See footnotes at end of table.

Parameter and units	Method	1974 EPA methods	14th ed. standard methods	References (page nos.) Pt. 31 1975 ASTM	References (page nos.) USGS methods [1]	Other approved methods
44. Cobalt—Total, milligrams per liter.	Digestion [14] followed by atomic absorption.[19]	107	148	345	80	[20] (37)
45. Cobalt—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total cobalt.					
46. Copper—Total, milligrams per liter.	Digestion [14] followed by atomic absorption [16] or by colorimetric (Neocuproine).	108	148 196	345 388	82 [3] (619)	[20] (37)
47. Copper—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total copper.					
48. Gold—Total, milligrams per liter.	Digestion [14] followed by atomic absorption.[19]					
49. Iridium—Total, milligrams per liter.	Digestion [14] followed by atomic absorption.[19]					
50. Iron—Total, milligrams per liter.	Digestion [14] followed by atomic absorption [16] or by colorimetric (Phenanthroline).	110	148 205	345 396	102	[3] (619)
51. Iron—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total iron.					
52. Lead—Total, milligrams per liter.	Digestion [14] followed by atomic absorption [16] or by colorimetric (Dithizone).	112	148 215	345	105	[3] (619)
53. Lead—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total lead.					
54. Magnesium—Total, milligrams per liter.	Digestion [14] followed by atomic absorption; or gravimetric.	114	148 221	345	109	[3] (619)
55. Magnesium—Dissolved milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total magnesium.					
56. Manganese—Total milligrams per liter.	Digestion [14] followed by atomic absorption [16] or by colorimetric (Persulfate or periodate).	116	148 235, 237	345	111	[3] (619)
57. Manganese—Dissolved milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total manganese.					
58. Mercury—Total, milligrams per liter.	Flameless atomic absorption.	118	156	338	[21] (51)	
59. Mercury—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total mercury.					
60. Molybdenum—Total, milligrams per liter.	Digestion [14] followed by atomic absorption.[19]	120		350		
61. Molybdenum—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total molybdenum.					
62. Nickel—Total, milligrams per liter.	Digestion [14] followed by atomic absorption [16] or by colorimetric (Heptoxime).	141	148	345	115	
63. Nickel—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total nickel.					
64. Osmium—Total, milligrams per liter.	Digestion [14] followed by atomic absorption.[19]					
65. Palladium—Total, milligrams per liter.	Digestion [14] followed by atomic absorption.[19]					
66. Platinum—Total, milligrams per liter.	Digestion [14] followed by atomic absorption.[19]					
67. Potassium—Total, milligrams per liter.	Digestion [14] followed by atomic absorption, colorimetric (Cobaltinitrite), or by flame photometric.	143	286 294	409	124	[3] (619)
68. Potassium—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total potassium.					
69. Rhodium—Total, milligrams per liter.	Digestion [14] followed by atomic absorption.[19]					
70. Ruthenium—Total, milligrams per liter.	Digestion [14] followed by atomic absorption.[19]					
71. Selenium—Total, milligrams per liter.	Digestion [14] followed by atomic absorption.[18] [19]	145	159			
72. Selenium—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total selenium.					
73. Silica—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by colorimetric (Molybdosilicate).	274	467	366	129	
74. Silver—Total,[22] milligrams per liter.	Digestion [14] followed by atomic absorption [16] or by colorimetric (Dithizone).	146	148 242		143	[3] (619) [20] (37)
75. Silver—Dissolved,[22] milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total silver.					
76. Sodium—Total, milligrams per liter.	Digestion [14] followed by atomic absorption or by flame photometric.	147	230	409	148	[3] (619)
77. Sodium—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total sodium.					

See footnotes at end of table

Parameter and units	Method	1974 EPA methods	14th ed. standard methods	References (page nos.) Pt. 31 1975 ASTM	USGS methods [1]	Other approved methods
78. Thallium—Total, milligrams per liter.	Digestion [16] followed by atomic absorption.[16]	140				
79. Thallium—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total thallium.					
80. Tin—Total, milligrams per liter.	Digestion [16] followed by atomic absorption.[16]	140			[11] (65)	
81. Tin—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total tin.					
82. Titanium—Total, milligrams per liter.	Digestion [16] followed by atomic absorption.[16]	151				
83. Titanium—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total titanium.					
84. Vanadium—Total, milligrams per liter.	Digestion [16] followed by atomic absorption [16] or by colorimetric (Gallic acid).	128	182 200	441	[11] (67)	
85. Vanadium—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total vanadium.					
86. Zinc—Total, milligrams per liter.	Digestion [45] followed by atomic absorption [16] or by colorimetric (Dithizone).	128	148 205	345	150	[3] (619) [10] (37)
87. Zinc—Dissolved, milligrams per liter.	0.45 micron filtration [17] followed by referenced method for total zinc.					
88. Nitrate (as N), milligrams per liter.	Cadmium reduction; brucine sulfate; automated cadmium or hydrazine reduction.[21]	201 197 207	422 427 430	308	119	[3] (614) [10] (38)
89. Nitrate (as N), milligrams per liter.	Manual or automated colorimetric (Diazotization).	215	434		121	
90. Oil and grease, milligrams per liter.	Liquid-liquid extraction with trichloro-trifluoroethane-gravimetric.	220	515			
91. Organic carbon; total (TOC), milligrams per liter.	Combustion—Infrared method.[22]	236	532	467	[10] (4)	
92. Organic nitrogen (as N), milligrams per liter.	Kjeldahl nitrogen minus ammonia nitrogen.	175, 150	437		122	[3] (612, 614)
93. Orthophosphate (as P), milligrams per liter.	Manual or automated ascorbic acid reduction.	240 256	431 434	304	131	[3] (621)
94. Pentachlorophenol, milligrams per liter.	Gas chromatography [19]					
95. Pesticides, milligrams per liter.	do.[19]		555	530	[10] (34)	
96. Phenols, milligrams per liter.	Colorimetric, (4AAP)	241	563	545		
97. Phosphorus (elemental), milligrams per liter.	Gas chromatography [20]					
98. Phosphorus; total (as P), milligrams per liter.	Persulfate digestion followed by manual or automated ascorbic acid reduction.	249 264	429, 431 434	304	128	[3] (621)
RADIOLOGICAL						
99. Alpha—Total, pCi per liter.	Proportional or scintillation counter.		648	501 [12] [13] (75+-75)		
100. Alpha—Counting error, pCi per liter.	do.		648	504	[13] (79)	
101. Beta—Total, pCi per liter.	Proportional counter		648	501 [12] [13] (75+-75)		
102. Beta—Counting error, pCi per liter.	do.		648	506	[13] (79)	
103. (a) Radium—Total, pCi per liter.	do.		661	661		
(b) ^{226}Ra, pCi per liter.	Scintillation counter.		667		[13] (31)	
RESIDUE						
104. Total, milligrams per liter.	Gravimetric, 103 to 105° C.	270	91			
105. Total dissolved (filterable), milligrams per liter.	Glass fiber filtration, 180° C.	268	92			
106. Total suspended (nonfilterable), milligrams per liter.	Glass fiber filtration, 103 to 105° C.	268	94			
107. Settleable, milliliters per liter or milligrams per liter.	Volumetric or gravimetric		96			
108. Total volatile, milligrams per liter.	Gravimetric, 550° C.	272	95			
109. Specific conductance, micromhos per centimeter at 25° C.	Wheatstone bridge conductimetry.	275	71	130	148	[3] (606)
110. Sulfate (as SO_4), milligrams per liter.	Gravimetric; turbidimetric; or automated colorimetric (barium chloranilate).	277 279	416 426	434 435		[3] (624) [3] (626)
111. Sulfide (as S), milligrams per liter.	Titrimetric—Iodine for levels greater than 1 mg per liter; Methylene blue photometric.	284	505 508		154	
112. Sulfite (as SO_3), milligrams per liter.	Titrimetric, iodine-iodate	286	508	426		
113. Surfactants, milligrams per liter.	Colorimetric (Methylene blue).	157	600	404	[10] (11)	
114. Temperature, degrees C.	Calibrated glass or electrometric thermometer.	286	126		[10] (31)	
115. Turbidity, NTU.	Nephelometric.	286	133	220	180	

[1] Recommendations for sampling and preservation of samples according to parameter measured may be found in "Methods for Chemical Analysis of Water and Wastes, 1974" U.S. Environmental Protection Agency, table 2, pp. viii-xii.

[1] All page references for USGS methods, unless otherwise noted, are to Brown, E., Skougstad, M. W., and Fishman, M. J., "Methods for Collection and Analysis of Water Samples for Dissolved Minerals and Gases," U.S. Geological Survey Techniques of Water-Resources Inv., book 5, ch. A1, (1970).

[2] EPA comparable method may be found on indicated page of "Official Methods of Analysis of the Association of Official Analytical Chemists" methods manual, 12th ed. (1975).

[4] Manual distillation is not required if comparability data on representative effluent samples are on company file to show that this preliminary distillation step is not necessary; however, manual distillation will be required to resolve any controversies.

[5] The method used must be specified.

[6] The 5 tube MPN is used.

[7] Skougstad, M. W. and others, "Methods for Collection and Analysis of Aquatic Biological and Mircobiological Samples: U.S. Geological Survey Techniques of Water-Resources Inv. book 5, ch. A4 (1973)."

[8] Since the membrane filter technique usually yields low and variable recovery from chlorinated wastewaters, the MPN method will be required to resolve any controversies.

[9] Adequately tested methods for benzidine are not available. Until approved methods are available, the following interim method can be used for the estimation of benzidine: (1) "Method for Benzidine and Its Salts in Wastewaters," available from Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268.

[10] American National Standard on Photographic Processing Effluents, Apr. 2, 1975. Available from ANSI, 1430 Broadway, New York, N.Y. 10018.

[11] Fishman, M. J. and Brown, Eugene, "Selected Methods of the U.S. Geological Survey for Analysis of Wastewaters," (1976) open-file report 76-177.

[12] Procedures for pentachlorophenol, chlorinated organic compounds, and pesticides can be obtained from the Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268.

[13] Color method (ADMI procedure) available from Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268.

[14] For samples suspected of having thiocyanate interference, magnesium chloride is used as the digestion catalyst. In the approved test procedure for cyanides, the recommended catalysis are replaced with 30 ml of a solution of 510 g/l magnesium chloride ($MgCl_2 \cdot 6H_2O$). This substitution will eliminate thiocyanate interference for both total cyanide and cyanide amenable to chlorination measurements.

[15] For the determination of total metals the sample is not filtered before processing. Because vigorous digestion procedures may result in a loss of certain metals through precipitation, a less vigorous treatment is recommended as given on p. 89 (4.1.4) of "Methods for Chemical Analysis of Water and Wastes" (1974). In those instances where a more vigorous digestion is desired the procedure on p. 82 (4.1.3) should be followed. For the measurement of the noble metal series (gold, iridium, osmium, palladium, platinum, rhodium and ruthenium), an aqua regia digestion is to be substituted as follows: Transfer a representitive aliquot of the well-mixed sample to a Griffin beaker and add 3 ml of concentrated redistilled HNO_3. Place the beaker on a steam bath and evaporate to dryness. Cool the beaker and cautiously add a 5 ml portion of aqua regia. (Aqua regia is prepared immediately before use by carefully adding 3 volumes of concentrated HCl to one volume of concentrated HNO_3.) Cover the beaker with a watch glass and return to the steam bath. Continue heating the covered beaker for 50 min. Remove cover and evaporate to dryness. Cool and take up the residue in a small quantity of 1:1 HCl. Wash down the beaker walls and watch glass with distilled water and filter the sample to remove silicates and other insoluble material that could clog the atomizer. Adjust the volume to some predetermined value based on the expected metal concentration. The sample is now ready for analysis.

[16] As the various furnace devices (flameless AA) are essentially atomic absorption techniques, they are considered to be approved test methods. Methods of standard addition are to be followed as noted in p. 78 of "Methods for Chemical Analysis of Water and Wastes," 1974.

[17] Dissolved metals are defined as those constitutents which will pass though a 0.45 μm membrane filter. A prefiltration is permissible to free the sample from larger suspended solids. Filter the sample as soon as practical after collection using the first 50 to 100 ml to rinse the filter flask. (Glass or plastic filtering apparatus are recommended to avoid possible contamination.) Discard the portion used to rinse the flask and collect the required volume of filtrate. Acidify the filtrate with 1:1 redistilled HNO_3 to a pH of 2. Normally, 3 ml of (1:1) acid per liter should be sufficient to preserve the samples.

[18] See "Atomic Absorption Newsletter," vol. 13, 75 (1974). Available from Perkin-Elmer Corp., Main Ave., Norwalk, Conn. 06852.

[19] Method available from Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268.

[20] Recommended methods for the analysis of silver in industrial wastewaters at concentrations of 1 mg/l and above are inadequate where silver exists as an inorganic halide. Silver halides such as the bromide and chloride are relatively insoluble in reagents such as nitric acid but are readily soluble in an aqueous buffer of sodium thiosulfate and sodium hydroxide to a pH of 12. Therefore, for levels of silver above 1 mg/l 20 ml of sample should be diluted to 100 ml by adding 40 ml each of 2M $Na_2S_2O_3$ and 2M NaOH. Standards should be prepared in the same manner. For levels of silver below 1 mg/l the recommended method is satisfactory.

[21] An automated hydrazine reduction method is available from the Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268.

[22] A number of such systems manufactured by various companies are considered to be comparable in their performance. In addition, another technique, based on combustion-methane detection is also acceptable.

[23] Goerlitz, D., Brown, E., "Methods for Analysis of Organic Substances in Water": U.S. Geological Survey Techniques of Water-Resources Inv., book 5, ch. A3 (1972).

[24] R. F. Addison and R. G. Ackman, "Direct Determination of Elemental Phosphorus by Gas-Liquid Chromatography," "Journal of Chromatography," vol. 47, No. 3, pp. 421-426, 1970.

[25] The method found on p. 75 measures only the dissolved portion while the method on p. 78 measures only suspended. Therefore, the 2 results must be added together to obtain "total."

[26] Stevens, H. H., Ficke, J. F., and Smoot, G. F., "Water Temperature—Influential Factors, Field Measurement and Data Presentation: U.S. Geological Survey Techniques of Water Resources Inv., book 1 (1975)."

4. In § 136.4, the second sentence of paragraph (c) is amended by deleting the word "subchapter" immediately following the phrase "procedure under this" and immediately preceding the word "shall" and replaced with the phrase "paragraph c;" and § 136.4 is amended by adding a new paragraph (d) to read as follows:

§ 136.4 Application for alternate test procedures.

* * * * *

(c) * * * Any application for an alternate test procedure under this paragraph (c) shall: * * *

(d) An application for approval of an alternate test procedure for nationwide use may be made by letter in triplicate to the Director, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268. Any application for an alternate test procedure under this paragraph (d) shall:

(1) Provide the name and address of the responsible person or firm making the application.

(2) Identify the pollutant(s) or parameter(s) for which nationwide approval of an alternate testing procedure is being requested.

(3) Provide a detailed description of the proposed alternate procedure, together with references to published or other studies confirming the general applicability of the alternate test procedure to the pollutant(s) or parameter(s) in waste water discharges from representative and specified industrial or other categories.

(4) Provide comparability data for the performance of the proposed alternate test procedure compared to the performance of the approved test procedures.

§ 136.5 [Amended]

5. In § 136.5, paragraph (a) is amended by inserting the phrase "proposed by the responsible person or firm making the discharge" immediately after the words "test procedure" and before the period that ends the paragraph.

6. In § 136.5, paragraph (b) is amended by inserting in the first sentence the phrase "proposed by the responsible person or firm making the discharge" immediately after the words "such application" and immediately before the comma. The second sentence of paragraph (b) is amended by deleting the phrase "Methods Development and Quality Assurance Research Laboratory" immediately after the phrase "State Permit Program and to the Director of the" at the end of the sentence, and inserting in its place the phrase "Environmental Monitoring and Support Laboratory, Cincinnati."

7. In § 136.5, paragraph (c) is amended by inserting the phrase "proposed by the responsible person or firm making the discharge" immediately after the phrase "application for an alternate test procedure" and immediately before the comma; and by deleting the phrase "Methods Development and Quality Assurance Laboratory" immediately after the phrase "application to the Director of the" and immediately before the phrase "for review and recommendation" and inserting in its place the phrase "Environmental Monitoring and Support Laboratory, Cincinnati."

8. In § 136.5, the first sentence of paragraph (d) is amended by inserting the phrase, "proposed by the responsible person or firm making the discharge," immediately after the phrase, "application for an alternate test procedure," and immediately before the comma.

The second sentence of paragraph (d) is amended by deleting the phrase, "Methods Development and Quality Assurance Research Laboratory," immediately after the phrase, "to the Regional Administrator by the Director of the," and immediately preceding the period ending the sentence and inserting in its place the phrase, "Environmental Monitoring and Support Laboratory, Cincinnati."

The third sentence of paragraph (d) is amended by deleting the phrase, "Methods Development and Quality Assurance Research Laboratory," immediately after the phrase, "forwarded to the Director," and immediately before the second comma and by inserting in its place the phrase, "Environmental Monitoring and Support Laboratory, Cincinnati."

9. Section 136.5 is amended by the addition of a new paragraph (e) to read as follows:

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§ 136.5 Approval of alternate test procedures.

* * * * *

(e) Within ninety days of the receipt by the Director of the Environmental Monitoring and Support Laboratory, Cincinnati of an application for an alternate test procedure for nationwide use, the Director of the Environmental Monitoring and Support Laboratory, Cincinnati shall notify the applicant of his recommendation to the Administrator to approve or reject the application, or shall specify additional information which is required to determine whether to approve the proposed test procedure. After such notification, an alternate method determined by the Administrator to satisfy the applicable requirements of this part shall be approved for nationwide use to satisfy the requirements of this subchapter; alternate test procedures determined by the Administrator not to meet the applicable requirements of this part shall be rejected. Notice of these determinations shall be submitted for publication in the FEDERAL REGISTER not later than 15 days after such notification and determination is made.

[FR Doc.76-35082 Filed 11-30-76;8:45 am]

FEDERAL REGISTER, VOL. 41, NO. 232--WEDNESDAY, DECEMBER 1, 1976, pp. 52780-52786

PART 136--GUIDELINES ESTABLISHING TEST PROCEDURES FOR THE ANALYSIS OF POLLUTANTS

Amendment of Regulations; Corrections*

Page	Parameter Number	Correction or Addition
52783	62, Nickel	14th ed. Standard Methods - add "232" to page reference opposite the colorimetric method designation.
52784	89	Parameter and units, change "Nitrate" to "Nitrite"
52784	96, Phenols	Delete the present method designation, "Colorimetric, (4 AAP)," and replace it with the method designation, "Distillation followed by colorimetric, (4 AAP)."
52784	96, Phenols	14th ed. Standard Methods, change "582" to "574"

Amendment; Correction**

52784	106, Total Suspended Residue	In "Method" column, after "103 to 105°C," add: "Glass fiber filtration, 103-105°C, post-washing of residue." Opposite this entry, in the "other Approved Methods" column, add (537)[27]. Then, on page 52785, add footnote 27 to read: "27. Standard Methods for the Examination of Water and Wastewater, 13th Edition (1971)."

*Federal Register, Volume 42, No. 12, Tuesday, January 18, 1977, pp. 3306-3307
**Federal Register, Volume 42, No. 139, Wednesday, July 20, 1977, p. 37205

NPDES/CERTIFICATIONS
ALTERNATIVE TEST PROCEDURES FOR LIMITED USE

TOPIC: Application and approval for individual permit-holders to use alternate test procedures (alternate to those published in the Federal Register for NPDES/certification purposes) for specific discharges.

SOURCE: Federal Register, Title 40, Chapter I, Subchapter D, Part 136:
- Vol 38, No. 199, October 16, 1973
- Vol 41, No. 232, December 1, 1976

A. Application for Alternate Test Procedures

The responsible person or firm making the discharge applies to the EPA Regional Administrator (RA) in the Region where the discharge occurs, through the Director of the State agency having permit-issuing authority. If the state does not issue permits, the application is sent directly to the EPA RA. One must:

1. Provide identifying information, i.e., name, address, permit number, etc.

2. Identify the pollutant or parameter involved.

3. Provide justification for using alternate procedure rather than stipulated test.

4. Provide a detailed description of the proposed procedure with references regarding the applicability to the effluents in question.

B. Approval of Alternate Test Procedures

An EPA Regional Administrator (RA) has the final responsibility for approval.

1. The State Director conducts a technical and administrative review and forwards the application and his recommendation to the RA.

2. The RA conducts a technical and administrative review.

   a. If the State Director recommended rejection for scientific and technical reasons, the RA denies the application and sends a copy of the rejected application and his decision to the applicant, the State Director and to the Director of the Environmental Monitoring and Support Laboratory (EMSL).

   b. Before approving any application, the RA sends a copy of the application to the Director of EMSL for review and recommendation.

3. Prior to 90 days of receipt of the application by the RA, the Director of EMSL forwards to the RA a recommendation providing the scientific and other technical basis for acceptance or rejection of the application.

4. Within 90 days of receipt, the RA notifies the applicant and the appropriate State agency of approval or rejection, or else specifies additional information required for the decision.

5. A copy of all approval and rejection notifications are sent to EMSL for purposes of national coordination.

NPDES/CERTIFICATIONS
ALTERNATIVE TEST PROCEDURES FOR NATIONWIDE USE

TOPIC: Application and approval by any person, laboratory, manufacturer, etc., for nationwide use of an alternate test procedure (alternate to those published in the Federal Register for NPDES/Certification purposes).

SOURCE: Federal Register, Title 40, Chapter I, Subchapter D, Part 136, Vol 41, No. 232, December 1, 1976

A. Application for Alternate Test Procedures

Any interested person, laboratory, manufacturer, etc., applies to the Director, Environmental Monitoring and Support Laboratory (EMSL), Cincinnati, Ohio 45268. One must:

1. Provide identifying information, i.e., name and address of the responsible person or firm.

2. Identify the pollutant(s) or parameter(s) involved.

3. Provide a detailed description of the proposed procedure with references regarding its applicability.

4. Provide comparability data (proposed procedure compared to procedure published in the Federal Register).

B. Approval of Alternate Test Procedures

The Administrator of the U.S. Environmental Protection Agency has the final responsibility for approval.

1. The Director of EMSL conducts a technical review.

2. Within 90 days of receipt, the Director of EMSL notifies the Administrator of his recommendation to approve or reject the application, or else returns the application for additional information required for the decision.

3. After notification of the EMSL recommendations, the Administrator determines whether or not the alternate test procedures meet the requirements set forth in the Federal Register, i.e., whether the procedures are to be approved or rejected.

4. Within fifteen days of the notification and determination, notice of the final decision is submitted to the Federal Register for publication.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
CINCINNATI, OHIO 45268

DATE: September 20, 1978

SUBJECT: Use of Chemical Test Kits for Compliance Monitoring

FROM: Dwight G. Ballinger, Director
Environmental Monitoring and Support Laboratory - Cincinnati

TO: Regional Quality Assurance Coordinators

A number of Regional Coordinators and Permit staff have received inquiries concerning the use of test kits for the parameters required by the Effluent Guidelines and Compliance Monitoring Sections of PL 92-500. This memorandum defines the position of EMSL on these test methods and contains recommendations for regional and state response to inquiries concerning acceptance.

Our interpretation of Section 304(g), implemented as "Guidelines Establishing Test Procedures for Analysis of Pollutants," in Federal Register, Dec. 1, 1976, is that the test kits are not equivalent to the procedures promulgated in the Federal Register and therefore are subject to the requirements governing alternate procedures. Therefore, it is necessary for the applicant to request the use of these test kits from the appropriate Regional Administrator as presented in the regulations. This recommendation is based upon the following factors:

SAMPLE PREPARATION

Most of the procedures selected for implementation of Section 304(g) are designed to measure the total constituent or element present in the sample or are specifically modified to determine a precisely defined form of the substance. The test kits considered herein generally do not utilize the necessary digestion or pretreatment required to measure the total constituent. In many cases, such as preliminary digestion with acids or distillations, these pretreatments cannot be performed satisfactorily under field conditions. Thus, the final result reported, when a kit is used, is nearly always less than the true value and acceptance of such data will lead to wrong interpretation of effluent loadings.

INTERFERENCES

It should be recognized that a majority of these test kit measurement techniques were developed for field use on domestic water supplies or relatively clean ambient streams and are not designed to provide for removal of interferences often encountered in municipal and industrial waste effluents.

MEASUREMENT SYSTEMS

In some cases the physical and chemical measurement principles differ from those employed in the reference methods. In addition, proprietary reagents of unknown composition are often provided with little or no information available on the reactions involved. Even though some of the tests are "based on Standard Methods," a number of factors rule out the acceptance of the test procedures as equivalent.

REAGENTS

In most cases the reagent concentrations are not the same as those described in the reference method. Volumes are generally pre-measured under unknown conditions and addition to the sample is often by means of inaccurate droppers or pipets. In short, the quality and quantity of reagents is not under the direct control of the analyst, as required by good analytical technique.

CALIBRATION AND COLOR MEASUREMENT

In most test kits a comparatively simple photometer is provided or available light is used to measure color intensity. Calibration scales are supplied by the manufacturer based upon factors developed under ideal conditions in his laboratory, and recalibration is difficult or not recommended. No provision is made for changes in reagent composition due to inadequate quality control in manufacturing or adverse storage conditions. The photometers usually available will not accept cells of sufficient path length to achieve the required sensitivity, and sealed standards incorporated in the kits are subject to changes in color with time. Finally, the band pass of these photometers is generally too wide for accurate measurement of the appropriate wavelength.

DATA REQUIREMENTS FOR ALTERNATE TEST PROCEDURES

When consideration of the above factors are not sufficient to reject an application for use of test kits, a request should be made for

comparative data upon which to judge the applicability of the alternative procedures. The attached protocol is recommended in developing the necessary information. The regulations require that the alternate procedures be used on the waste being monitored and that application be made to the Regional Administrator or State Director having jurisdiction over the permit issuance.

It is recognized that under unusual conditions the applicant may have difficulty obtaining comparative data because of lack of laboratory facilities at remote locations. Even in these cases, the applicant should be urged to obtain adequate data by having the necessary work done at a base laboratory or on contract. The acceptance of alternate procedures without supporting laboratory results may significantly weaken the pollution control efforts intended by PL 92-500.

The use of field test kits, as with other alternate procedures, should be considered on a case-by-case basis, with judgment based on all of the factors involved. No blanket acceptance of test methods will be recommended unless a large volume of data have been accumulated clearly showing that the use of the specific alternate procedure on a wide variety of sample types will provide test results equivalent in precision and accuracy to the reference methods. When such data are available, the method will probably be incorporated in amendments to the listing of Dec. 1, 1976, making further substantiation unnecessary.

Attachment

cc: Walter G. Gilbert, Director
National Training & Operational Technology Center

Attachment

DATA REQUIREMENTS
NATIONWIDE APPROVAL OF ALTERNATE TEST PROCEDURES

1) Five industrial (discharge) sources identified by Standard Industrial Classification (SIC) code or five drinking water sources,

2) Six samples from each source.

3) Four replicate analyses each by the proposed and approved method.

Sources		Samples		Replicates		Methods		Total
5	X	6	X	4	X	2	=	240

DATA REQUIREMENTS
LIMITED - USE APPROVAL OF ALTERNATE TEST PROCEDURE

STATE OR REGIONAL USE

1) Five sources.

2) Three samples from each source.

3) Four replicate analyses each by the proposed and approved method.

Sources		Samples		Replicates		Methods		Total
5	X	3	X	4	X	2	=	120

DATA REQUIREMENTS
LIMITED - USE APPROVAL OF ALTERNATE TEST PROCEDURE

PERMIT HOLDER OR DRINKING WATER SYSTEM

1) Three samples from each source.

2) Four replicate analyses each by the proposed and approved method.

Sources		Samples		Replicates		Methods		Total
1	X	3	X	4	X	2	=	24

STATISTICAL PROTOCOL
APPROVAL OF ALTERNATE TEST PROCEDURES

1) Calculate basic statistics of mean and standard deviation.

2) Test for outliers.

3) Frequency counts and histogram to check distribution.

4) Cochrans test for equality among within-sample standard deviation.

5) F-test for equality of pooled within-sample variances.

6) T-test for equality of method means.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
CINCINNATI, OHIO 45268

DATE: September 20, 1978

SUBJECT: Use of "Prepared" Reagents in NPDES Compliance

FROM: Dwight G. Ballinger, Director
Environmental Monitoring and Support
Laboratory - Cincinnati

TO: Regional Quality Assurance Coordinators

We encourage the use of pre-prepared solutions and standards by sewage treatment plant operators and others for their required compliance monitoring activities, providing such solutions have been prepared according to the reagents section of the approved methods cited in the Federal Register of December 1, 1976. We would, however, be opposed to the use of commercial reagents that are of unknown composition.

In allowing the use of known pre-prepared solutions and standards, we strongly recommend that the following quality control checks be observed to insure their validity:

1. Date all solutions upon receipt of shipment, store in separate, appropriate area, and observe stated shelf life.

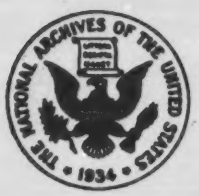
2. Verify that solutions and standards are valid by initially checking them against a quality control check sample available from EMSL through the appropriate Regional QA Coordinator. These check samples are available for most of the common measurements required in the NPDES. They are shipped in a sealed vial as a sample concentrate with the actual composition being provided in a separately sealed envelope.

3. Verify that these solutions are stable on a routine basis by periodically comparing them against a quality control check sample or a standard from another source.

cc: Walter G. Gilbert, Director
National Training & Operational Technology Center

federal register

WEDNESDAY, DECEMBER 24, 1975



PART IV:

ENVIRONMENTAL PROTECTION AGENCY

■

WATER PROGRAMS

National Interim Primary Drinking Water Regulations

Title 40—Protection of Environment

CHAPTER I—ENVIRONMENTAL PROTECTION AGENCY

SUBCHAPTER D—WATER PROGRAMS

[FRL 464-7]

PART 141—NATIONAL INTERIM PRIMARY DRINKING WATER REGULATIONS

On March 14, 1975, the Environmental Protection Agency (EPA) proposed National Interim Primary Drinking Water Regulations pursuant to sections 1412, 1414, 1415, and 1450 of the Public Health Service Act ("the Act"), as amended by the Safe Drinking Water Act ("SDWA," Pub. L. 93-523), 40 FR 11990. EPA held public hearings on the proposed regulations in Boston, Chicago, San Francisco, and Washington during the month of April. Several thousand pages of comments on the proposed regulations were received and evaluated. In addition, the Agency has received comments and information on the proposed regulations from the National Drinking Water Advisory Council, the Secretary of Health, Education, and Welfare, and from numerous others during meetings with representatives of State agencies, public interest groups and others.

The regulations deal only with the basic legal requirements. Descriptive material will be provided in a guidance manual for use by public water systems and the States.

The purpose of this preamble to the final regulations is to summarize the most significant changes made in the proposed regulations as a result of comments received and the further consideration of available information. A more detailed discussion of the comments and of changes in the proposed regulations is attached as Appendix A.

Water Systems Covered

The Safe Drinking Water Act applies to each "public water system," which is defined in Section 1401(4) of the Act as "a system for the provision to the public of piped water for human consumption, if such system has at least fifteen service connections or regularly serves at least twenty-five individuals." Privately owned as well as publicly owned systems are covered. Service "to the public" is interpreted by EPA to include factories and private housing developments. (See generally, House Report, pp. 16-17.)

The definition of "public water system" proposed in the Interim Primary Drinking Water Regulations sought to explain the meaning of the statutory reference to "regular" service. It was proposed to interpret this term as including service for as much as three months during the year. Because the proposed definition would have excluded many large campgrounds, lodges, and other public accommodations which serve large numbers of tourists but which are open for slightly less than three months each year, the definition in the final version covers systems serving an average of at least twenty-five individuals at least 60 days out of the year. The use of a minimum number of days rather than months also makes clear that a system may qualify as a public water system even if it is not open every day during a given month.

Once "public water system" has been defined, it is necessary to define the two major types of public water systems—those serving residents and those serving transients or intermittent users. The possible health effects of a contaminant in drinking water in many cases are quite different for a person drinking the water for a long period of time than for a person drinking the water only briefly or intermittently. Different regulatory considerations may in some cases apply to systems which serve residents as opposed to systems which serve transients or intermittent users. Accordingly, § 141.2(e) makes clear that all "public water systems" fall within either the category of "community water systems" or the category of "non-community water systems." To make clear which regulatory requirements apply to which type of system, the category covered is specifically indicated throughout the regulations.

The proposed regulations defined a "community water system" as "a public water system which serves a population of which 70 percent or greater are residents." Reliance in the proposed definition on the percentage of water system users who are residents would result in treating some fairly large resort communities with many year-round residents as non-community systems. Therefore, the definition of "community water system" has been changed to cover any system which serves at least 15 service connections used by year-round residents or serves at least 25 year-round residents.

Small Community Water Systems

Many community water systems in the country are quite small. Since it is the intention of the Act to provide basically the same level of health protection to residents of small communities as to residents of large cities, and since a number of advanced water treatment techniques are made feasible only by economies of scale, the cost of compliance with the requirements of the Act may pose a serious problem for many small communities. The regulations seek to recognize the financial problems of small communities by requiring more realistic monitoring for systems serving fewer than 1,000 persons. Variances and exemptions authorized by the Act can also assist in dealing with economic problems of small community systems in appropriate cases, at least temporarily. EPA will provide technical assistance on effective treatment techniques which can be used by small systems.

These methods of dealing with the financial problems of some small community systems may not be sufficient in specific instances to make compliance with all applicable regulatory requirements feasible. EPA is commencing a study of potential problems faced by small community systems in meeting applicable requirements under the Act and these regulations, and, if necessary, will make additional adjustments in the Interim Primary Drinking Water Regulations prior to their effective date.

Non-Community Systems

"Non-community systems" are basically those systems which serve transients. They include hotels, motels, restaurants, campgrounds, service stations, and other public accommodations which have their own water system and which have at least 15 service connections or serve water to a daily average of at least 25 persons. Some schools, factories and churches are also included in this category. It is conservatively estimated that there are over 200,000 non-community water systems in the country. However, it should be recognized that while their number is large, they normally are not the principal source of water for the people they serve.

The regulations as proposed would have applied all maximum contaminant levels to non-community systems as well as to community systems. This approach failed to take into account the fact that the proposed maximum contaminant levels for organic chemicals and most inorganic chemicals were based on the potential health effects of long-term exposure. Those levels are not necessary to protect transients or intermittent users. Therefore, the final regulations provide that maximum contaminant levels for organic chemicals, and for inorganic chemicals other than nitrates, are not applicable to non-community systems. An exception was made for nitrates because they can have an adverse health effect on susceptible infants in a short period of time.

Even without monitoring for organic chemicals or most inorganic chemicals, in the initial stages of implementation of the drinking water regulations, monitoring results from tens of thousands of non-community systems could overwhelm laboratory capabilities and other resources. This could delay effective implementation of the regulations with respect to the community systems which provide the water which Americans drink every day. To avoid this result, non-community systems will be given two years after the effective date of the regulations to commence monitoring. In the meantime, non-community systems which already monitor their water are encouraged to continue to do so, and the States are encouraged to take appropriate measures to test or require monitoring for non-community systems that serve large numbers of people.

Of course, non-community systems which pose a threat to health should be dealt with as quickly as possible. The maximum contaminant levels applicable to non-community water systems therefore will take effect 18 months after promulgation, at the same time as levels applicable to community systems. Inspection and enforcement authority will apply to non-community systems at the same time as to community systems.

Sanitary Surveys

EPA encourages the States to conduct sanitary surveys on a systematic basis.

These on-site inspections of water systems are more effective in assuring safe water to the public than individual tests taken in the absence of sanitary surveys. The regulations provide that monitoring frequencies for coliform bacteria can be changed by the entity with primary enforcement responsibility for an individual non-community system, and in certain circumstances for an individual community system, based on the results of a sanitary survey.

Maximum Contaminant Levels

Numerous comments were received by EPA on the substances selected for the establishment of maximum contaminant levels and on the levels chosen. Congress anticipated that the initial Interim Primary Drinking Water Regulations would be based on the Public Health Service Standards of 1962, and this Congressional intent has been followed. Comments received on the various levels did not contain new data sufficient to require the establishment of levels different from those contained in the Public Health Service Standards.

Water Consumption

The maximum contaminant levels are based, directly or indirectly, on an assumed consumption of two liters of water per day. The same assumption was used in the 1962 Standards. This assumption has been challenged because of instances where much higher water consumption rates occur. EPA's justification for using the two-liter figure is that it already represents an above average water or water-based fluid intake. Moreover, while the factor of safety may be somewhat reduced when greater quantities of water are ingested, the maximum contaminant levels based on the two-liter figure provide substantial protection to virtually all consumers. If, as has been suggested, a water consumption rate of eight liters per day is used as the basis for maximum contaminant level, all of the proposed MCL's would have to be divided by four, greatly increasing the monitoring difficulties, and in some cases challenging the sensitivity of accepted analytical procedures. It could be expected, in such a case, that the maximum contaminant levels would be exceeded to a significant degree, and that specialized treatment techniques would be required to order that the contaminant levels would be reduced. The economic impact of a move in this direction would be enormous. It is not technically or economically feasible to base maximum contaminant levels on unusually high consumption rates.

Safety Factors

A question was raised about the fact that different safety factors are contained in various maximum contaminant levels. The levels are not intended to have a uniform safety factor, at least partly because the knowledge of and the nature of the health risks of the various contaminants vary widely. The levels set are the result of experience, evaluation of the available data, and professional judgment. They have withstood the test of time and of professional review. They are being subjected to further review by the National Academy of Sciences in connection with development of data for the Revised Primary Drinking Water Regulations.

MCL's Based on Temperature

A question was also raised as to whether ranges of maximum contaminant levels should be established on the basis of the climate in the area served by the public water system, as was done with fluoride. EPA believes that the use of a temperature scale for fluoride is more appropriate than for other chemicals because of the studies available on the fluoride-temperature relationship and because there is a small margin with fluoride between beneficial levels and levels that cause adverse health effects.

MCL's Deleted

Three proposed maximum contaminant levels have been eliminated in the final regulations because they are not justified by the available data. One of these is carbon chloroform extract (CCE), which is discussed separately below. The others are the proposed levels for the standard bacterial plate count and cyanide. In the case of the plate count, it is believed that the coliform limits contained in the regulations, combined with the turbidity maximum contaminant level, adequately deal with bacterial contamination. However, EPA continues to believe that the standard plate count is a valid indicator of bacteriological quality of drinking water, and recommends that it be used in appropriate cases in conjunction with the coliform tests as an operational tool.

The proposed maximum contaminant level for cyanide was eliminated because the possibility of cyanide contamination can be effectively addressed only by the use of emergency action, such as under Section 1431 of the Act. EPA's 1969 Community Water Supply Study did not reveal a single instance in which cyanide was present in a water system at a level greater than one-thousandth of the level at which cyanide is toxic to humans.

Available data indicate that cyanide will be present in water systems at toxic levels only in the event of an accident, such as a spill from a barge collision. Maximum contaminant levels are not the appropriate vehicle for dealing with such rare, accidental contamination.

Heptachor, heptachlor epoxide and chlordane have also been removed from the list of maximum contaminant levels at least temporarily in view of the pending cancellation and suspension proceedings under the Federal Insecticide, Fungicide and Rodenticide Act involving those pesticides. When the results of these proceedings are available, EPA will again consider whether maximum contaminant levels should be established for those three pesticides.

Sodium and Sulfates

A number of comments were received on the potential health effects of sodium and sulfates. The National Drinking Water Advisory Council has recommended that consideration be given to the monitoring of these constituents, but has not recommended the adoption of maximum contaminant levels because available data do not support the adoption of any specific levels. EPA has requested the National Academy of Sciences to include sodium and sulfates among the contaminants to be studied by NAS, and to include information on the health effects of sodium and sulfates in the report to be made by NAS in December 1976.

Since a number of persons suffer from diseases which are influenced by dietary sodium intake and since there are others who wish to restrict their sodium intake, it is desirable that the sodium content of drinking water be known. Those affected can, by knowing the sodium concentration in their drinking water, make adjustments to their diets or, in extreme cases, seek alternative sources of water to be used for drinking and food preparation. It is recommended that the States institute programs for regular monitoring of the sodium content of drinking water served to the public, and for informing physicians and consumers of the sodium concentration in drinking water.

A relatively high concentration of sulfate in drinking water has little or no known laxative effect on regular users of the water, but transcients using such water sometimes experience a laxative effect. It is recommended that the States institute monitoring programs for sulfates, and that transients be notified if the sulfate content of the water is high. Such notification should include an assessment of the possible physiological effects of consumption of the water.

PCB's and Asbestos

An interagency comment expressed concern for asbestos and PCB's in the environment and noted the need for at least a monitoring requirement, if not for MCL's, for these contaminants. EPA is also concerned, but for the moment lacks sufficient evidence regarding analytical methods, health effects, or occurrence in the environment to establish MCL's. The Agency is conducting research and cooperating in research projects to develop criteria for establishing needed limits as quickly as possible. A monitoring study on a number of organic chemical contaminants, including PCB's, for which MCL's are not being established at this time, will be contained in an organic chemical monitoring regulation that is being promulgated with these regulations. Regarding asbestos, HEW and EPA are sponsoring a number of studies this year at an approximate cost of $16 million to establish health effects, anayltical methods and occurrence.

Point of Measurement

Other comments on maximum contaminant levels focused on the proposed requirement that such levels be tested at the consumer's tap. Concern was expressed over the inability of the public water system to control potential sources

of contaminants which are under the control of the consumer.

The promulgated definition of "maximum contaminant level," § 141.2(d), retains the requirement that the maximum contaminant level be measured at the tap except in the case of turbidity, which should be measured at the point of entry to the distribution system. However, the definition has been expanded to make clear that contaminants added to the water by circumstances under the control of the consumer are not the responsibility of the supplier of water, unless the contaminants result from corrosion of piping and plumbing resulting from the quality of the water supplied. It should be noted, however, that this requirement should not be interpreted as to discourage local, aggressive cross connection control measures.

Coliform Bacteria MCL's

The promulgated MCL's for coliform bacteria are basically the 1962 Public Health Service Standards, with minor refinements and clarifications. However, further changes may be desirable. For example, the MCL's for the membrane filter analytical method do not resolve the question of how many coliform bacteria are assumed to be present in a single highly contaminated sample. Some laboratories assume an upper limit of 50, while others seek to continue to count individual bacteria to a level of 100 or even higher in a single sample. The upper limit assumed will affect the monthly average which is calculated to determine compliance with the MCL's.

Another question relating to the coliform bacteria MCL's is the matter of possible spurious positive samples. As the regulations are written, all routine samples taken to determine compliance with the MCL's must be counted, regardless of the results of analysis of any check samples that may be taken. The reason for this is that bacterial contamination is often intermittent or transient, and as a result negative check samples taken a day or more after a positive sample cannot demonstrate that the positive result was in error. It may be possible, however, to prescribe a means of dealing with spurious positive results without compromising the integrity of the MCL's.

A third question concerning the MCL's for coliform bacteria is the relationship of monthly averages of coliform bacteria levels to monthly percentages of positive samples. For example, the monthly average MCL for the membrane filter method is violated if the monthly average exceeds one coliform bacterium per sample. However, for purposes of determining whether the monthly-percentage-of-positive-samples MCL is violated, a sample is counted as positive only if it contains more than four coliform bacteria. Thus, it is possible, particularly when a relatively small number of samples is taken, for a system to fail the monthly average MCL even when no single sample taken during the month is out of compliance with the limit.

These and other questions concerning the coliform bacteria MCL's will be reviewed further by EPA. If review indicates that changes in the MCL's are desirable, those changes will be made as soon as possible but within 6 months, in time to take effect at the same time as the initial Interim Primary Drinking Water Regulations.

Organic Chemicals

The proposed maximum contaminant levels for organic pesticides, other than the three which are the subject of cancellation and suspension proceedings, have been retained. It is anticipated that additional organic pesticides will be added to the regulations if surveys of pesticides in drinking water being conducted by EPA indicate that this is needed.

The proposed regulations also contained a maximum contaminant level for organic chemicals obtained by the carbon chloroform extract (CCE) method. It was anticipated by Congress that organic chemicals would be dealt with primarily in the Revised Primary Drinking Water Regulations because of the paucity of accurate data on the health effects of various organic chemicals, the large number of such chemicals, uncertainities over appropriate treatment techniques, and the need for additional information on the incidence of specific organic chemicals in drinking water supplies. EPA thought that the CCE standard might provide an appropriate means of dealing with organic chemicals as a class pending action on the Revised Primary Regulations.

The CCE standard was originally developed as a test for undesirable tastes and odors in drinking water. As concern developed over the health effects of organic chemicals, the possibility of using CCE as a health standard rather than an esthetic standard was considered.

As pointed out by numerous comments, CCE has many failings as an indicator of health effects of organic chemicals. To begin with, the test obtains information on only a fraction of the total amount of organic chemicals in the water sampled. Furthermore, there is serious question as to the reliability of CCE in identifying those organic chemicals which are most suspected of adverse health effects. In addition, there are no existing data on which a specific level for CCE can be established on a rational basis. To establish a maximum contaminant level under these circumstances would almost certainly do more harm than good. It could give a false sense of security to persons served by systems which are within the established level and a false sense of alarm to persons served by systems which exceed the level. It also would divert resources from efforts to find more effective ways of dealing with the organic chemicals problem.

EPA believes that the intelligent approach to the organic chemicals question is to move ahead as rapidly as possible along two fronts. First, EPA is adopting simultaneously with these regulations a Subpart E of Part 141, containing requirements for organic chemical monitoring pursuant to Sections 1445 and 1450 of the Act.

The regulations require that designated public water systems collect samples of raw and treated water for submission to EPA for organics analysis. EPA will analyze the samples for a number of broad organic parameters, including carbon chloroform extract (CCE), volatile and non-volatile total organic carbon (VTOC and NVTOC), total organic chlorine (TOCl), ultraviolet absorbancy, and fluorescence. In addition, monitoring will be required for probably 21 specific organic compounds. Selection of the specific compounds has been based on the occurrence or likelihood of occurrence in treated water, toxicity data and availability of practical analytical methods. Laboratory analyses will be used to evaluate the extent and nature of organic chemical contamination of drinking water, to evaluate the validity of the general organic parameters as surrogates for measures of harmful organic chemicals, and to determine whether there is an adequate basis for establishing maximum contaminant levels for specific organics or groups of organics.

Second, EPA is embarking on an intensive research program to find answers to the following four questions:

1. What are the effects of commonly occurring organic compounds on human health?

2. What analytical procedures should be used to monitor finished drinking water to assure that any Primary Drinking Water Regulations dealing with organics are met?

3. Because some of these organic compounds are formed during water treatment, what changes in treatment practices are required to minimize the formation of these compounds in treated water?

4. What treatment technology must be applied to reduce contaminant levels to concentrations that may be specified in the Primary Drinking Water Regulations?

This research will involve health-effects and epidemiological studies, investigations of analytical methodology, and pilot plant and field studies of organic removal unit processes. Some phases of the research are to be completed by the end of this year, while much of the remainder are to be completed within the next calendar year.

As soon as sufficient information is derived from the monitoring program and related research, the Interim Primary Drinking Water Regulations will be amended so that the organic chemicals problem can be dealt with without delay. The monitoring process will be completed within 1 year.

During the interim period, while satisfactory MCL's for organic contamination in drinking water are being developed, EPA will act in specific cases where appropriate to deal with organic contamination. If the EPA monitoring program reveals serious specific cases of contamination, EPA will work with State and local authorities to identify the source and nature of the problem and to

take remedial action. EPA will also aid the States in identifying additional community water supplies that require analysis.

PUBLIC NOTICE

The public notice requirements proposed in § 141.32 did not distinguish between community and non-community public water systems. They would have required that public notice of non-compliance with applicable regulations be made by newspaper, in water bills, and by other media for all public water systems. These requirements are inappropriate and ineffective in the case of most non-community water systems. Those systems principally serve transients who do not receive water bills from the system and who probably are not exposed significantly to the local media. A more effective approach would be to require notice that can inform the transient before he drinks the system's water, and thereby both warn the transient and provide an incentive to the supplier of water to remedy the violation. Accordingly, Section 141.32 as adopted provides that in the case of non-community systems, the entity with primary enforcement responsibility shall require that notice be given in a form and manner that will insure that the public using the public water system is adequately informed.

The proposed public notice requirements also failed to distinguish between different types of violations of the Interim Primary Drinking Water Regulations. Since the urgency and importance of a notice varies according to the nature of the violation involved, § 141.32 as promulgated seeks to match the type of notice required with the type of violation involved. Written notice accompanying a water bill or other direct notice by mail is required for all violations of the regulations, including violations of monitoring requirements, and for the grant of a variance or exemption. In addition, notice by newspaper and notification to radio and television stations is required whenever a maximum contaminant level is exceeded, or when the entity with primary enforcement responsibility requires such broader notice.

QUALITY CONTROL AND TESTING PROCEDURES

Section 1401(1) of the Act defines "primary drinking water regulation" to include "quality control and testing procedures." The promulgated regulations include testing requirements for each maximum contaminant level, including check samples and special samples in appropriate cases. The regulations also specify the procedures to be followed in analyzing samples for each of the maximum contaminant levels. These procedures will be updated from time to time as advances are made in analytical methods. For example, references to "Standard Methods for the Examination of Water and Wastewater" are to the current, 13th, edition, but these references will be changed to cite the 14th edition when it is available in the near future.

A key element of quality control for public water systems is accurate laboratory analysis. Section 141.28 of the regulations provides that analyses conducted for the purpose of determining compliance with maximum contaminant levels must be conducted by a laboratory approved by the entity with primary enforcement responsibility. EPA will develop as soon as possible, in cooperation with the States and other interested parties, criteria and procedures for laboratory certification. A State with primary enforcement responsibility will have a laboratory certified by EPA pursuant to the prescribed criteria and procedures, and in turn will certify laboratories within the State.

Record-keeping requirements and reports to the State also will assist in quality control efforts.

RECORD-KEEPING

Adequate record-keeping is necessary for the proper operation and administration of a public water system. It is also important for providing information to the public, providing appropriate data for inspection and enforcement activities and providing information on which future regulations can be based. Accordingly, a new § 141.33 has been added to the regulations to require that each public water system maintain records of sample analyses and of actions to correct violations of the Primary Drinking Water Regulations.

ECONOMIC AND COST ANALYSIS

A comprehensive economics study has been made of the Interim Primary Drinking Water Regulations. This study estimates the costs of the regulations, evaluates the potential economic impact, and considers possible material and labor shortages. The results of this analysis are summarized here.

Total investment costs to community water systems to achieve compliance with these regulations are estimated to be between $1,050 and $1,765 million. It is estimated that non-community systems will invest an additional $24 million. The range of the estimate is due to uncertainty as to the design flow that will be used in installing treatment facilities. Systems not in compliance will have to consider sizing their new components to reflect average daily flow conditions, or maximum daily flow conditions in cases where system storage is not adequate.

This investment will be spread over several years. Investor-owned systems will bear about one-fourth of these costs, and publicly-owned systems the remainder. It is not anticipated that systems will have difficulty financing these capital requirements.

In annual terms, national costs are expected to be within the following ranges:

	In millions
Capital costs	$146–247
Operations and maintenance	263–263
Monitoring (routine only)	17– 35
Total	$426–545

Although these aggregate figures are large, most water consumers will not be significantly affected. For those users in systems serving 10,000 persons or more, the average annual treatment cost per capita may increase from less than $1.00 for systems requiring disinfection and lead control, to between $15 to $35 for control of turbidity and heavy metal removal. For systems serving less than 100 persons, the average annual per capita costs of disinfection, lead control and fluoride/arsenic removal are estimated to be between $2.10 and $11.80. However, if turbidity control or heavy metal removal were required in a system of this size then costs are expected to range from $52 to $237 per year per capita. EPA is aware of the serious potential economic impact on users in these small systems. However, the legislative history specifies that the regulations should be based on costs that can be reasonably afforded by large metropolitan or regional systems. Further economic evaluation of these systems is being conducted, and realistic options for these small systems are being reviewed. Options that will be under consideration include less costly treatment technologies; formation of regional systems; and use of alternative water sources. Industrial and commercial users, whether providing their own water or using public systems, are not expected to be significantly affected by these regulations.

Possible constraints to the implementation of the interim primary regulations were examined. Although there will be an increase in demand for chemicals, manpower, laboratories, and construction of treatment facilities, it is not anticipated that any of these factors will be a serious obstacle to implementation of these regulations over a reasonable time frame.

For the reasons given above, Chapter 40 of the Code of Federal Regulations is hereby amended by the addition of the following new Part 141. These regulations will take effect 18 months after promulgation.

(It is hereby certified that the economic and inflationary impacts of these regulations have been carefully evaluated in accordance with Executive Order 11821)

Dated: December 10, 1975.

RUSSELL E. TRAIN,
Administrator.

Subpart A—General

Sec.
141.1 Applicability.
141.2 Definitions.
141.3 Coverage.
141.4 Variances and exemptions.
141.5 Siting requirements.
141.6 Effective date.

Subpart B—Maximum Contaminant Levels

141.11 Maximum contaminant levels for inorganic chemicals.
141.12 Maximum contaminant levels for organic chemicals.
141.13 Maximum contaminant levels for turbidity.
141.14 Maximum microbiological contaminant levels.

Subpart C—Monitoring and Analytical Requirements

141.21 Microbiological contaminant sampling and analytical requirements.

Sec.
141.22 Turbidity sampling and analytical requirements.
141.23 Inorganic chemical sampling and analytical requirements.
141.24 Organic chemical sampling and analytical requirements.
141.27 Alternative analytical techniques.
141.28 Approved laboratories.
141.29 Monitoring of consecutive public water systems.

Subpart D—Reporting, Public Notification, and Record Keeping

141.31 Reporting requirements.
141.32 Public notification of variances, exemptions, and non-compliance with regulations.
141.33 Record maintenance.

AUTHORITY: Secs. 1412, 1414, 1445, and 1450 of the Public Health Service Act, 88 Stat. 1660 (42 U.S.C. 300g-1, 300g-3, 300j-4, and 300j-9).

Subpart A—General

§ 141.1 Applicability.

This part establishes primary drinking water regulations pursuant to section 1412 of the Public Health Service Act, as amended by the Safe Drinking Water Act (Pub. L. 93-523); and related regulations applicable to public water systems.

§ 141.2 Definitions.

As used in this part, the term:

(a) "Act" means the Public Health Service Act, as amended by the Safe Drinking Water Act, Pub. L. 93-523.

(b) "Contaminant" means any physical, chemical, biological, or radiological substance or matter in water.

(c) "Maximum contaminant level" means the maximum permissible level of a contaminant in water which is delivered to the free flowing outlet of the ultimate user of a public water system, except in the case of turbidity where the maximum permissible level is measured at the point of entry to the distribution system. Contaminants added to the water under circumstances controlled by the user, except those resulting from corrosion of piping and plumbing caused by water quality, are excluded from this definition.

(d) "Person" means an individual, corporation, company, association, partnership, State, municipality, or Federal agency.

(e) "Public water system" means a system for the provision to the public of piped water for human consumption, if such system has at least fifteen service connections or regularly serves an average of at least twenty-five individuals daily at least 60 days out of the year. Such term includes (1) any collection, treatment, storage, and distribution facilities under control of the operator of such system and used primarily in connection with such system, and (2) any collection or pretreatment storage facilities not under such control which are used primarily in connection with such system. A public water system is either a "community water system" or a "non-community water system."

(i) "Community water system" means a public water system which serves at least 15 service connections used by year-round residents or regularly serves at least 25 year-round residents.

(ii) "Non-community water system" means a public water system that is not a community water system.

(f) "Sanitary survey" means an on-site review of the water source, facilities, equipment, operation and maintenance of a public water system for the purpose of evaluating the adequacy of such source, facilities, equipment, operation and maintenance for producing and distributing safe drinking water.

(g) "Standard sample" means the aliquot of finished drinking water that is examined for the presence of coliform bacteria.

(h) "State" means the agency of the State government which has jurisdiction over public water systems. During any period when a State does not have primary enforcement responsibility pursuant to Section 1413 of the Act, the term "State" means the Regional Administrator, U.S. Environmental Protection Agency.

(i) "Supplier of water" means any person who owns or operates a public water system.

§ 141.3 Coverage.

This part shall apply to each public water system, unless the public water system meets all of the following conditions:

(a) Consists only of distribution and storage facilities (and does not have any collection and treatment facilities);

(b) Obtains all of its water from, but is not owned or operated by, a public water system to which such regulations apply;

(c) Does not sell water to any person; and

(d) Is not a carrier which conveys passengers in interstate commerce.

§ 141.4 Variances and exemptions.

Variances or exemptions from certain provisions of these regulations may be granted pursuant to Sections 1415 and 1416 of the Act by the entity with primary enforcement responsibility. Provisions under Part 142, *National Interim Primary Drinking Water Regulations Implementation*—subpart E (Variances) and subpart F (Exemptions)—apply where EPA has primary enforcement responsibility.

§ 141.5 Siting requirements.

Before a person may enter into a financial commitment for or initiate construction of a new public water system or increase the capacity of an existing public water system, he shall notify the State and, to the extent practicable, avoid locating part or all of the new or expanded facility at a site which:

(a) Is subject to a significant risk from earthquakes, floods, fires or other disasters which could cause a breakdown of the public water system or a portion thereof; or

(b) Except for intake structures, is within the floodplain of a 100-year flood or is lower than any recorded high tide where appropriate records exist.

The U.S. Environmental Protection Agency will not seek to override land use decisions affecting public water systems siting which are made at the State or local government levels.

§ 141.6 Effective date.

The regulations set forth in this part shall take effect 18 months after the date of promulgation.

Subpart B—Maximum Contaminant Levels

§ 141.11 Maximum contaminant levels for inorganic chemicals.

(a) The maximum contaminant level for nitrate is applicable to both community water systems and non-community water systems. The levels for the other inorganic chemicals apply only to community water systems. Compliance with maximum contaminant levels for inorganic chemicals is calculated pursuant to § 141.23.

(b) The following are the maximum contaminant levels for inorganic chemicals other than fluoride:

Contaminant	*Level, milligrams per liter*
Arsenic	0.05
Barium	1.
Cadmium	0.010
Chromium	0.05
Lead	0.05
Mercury	0.002
Nitrate (as N)	10.
Selenium	0.01
Silver	0.05

(c) When the annual average of the maximum daily air temperatures for the location in which the community water system is situated is the following, the maximum contaminant levels for fluoride are:

Temperature Degrees Fahrenheit	Degrees Celsius	Level, milligrams per liter
53.7 and below	12.0 and below	2.4
53.8 to 58.3	12.1 to 14.6	2.2
58.4 to 63.8	14.7 to 17.6	2.0
63.9 to 70.6	17.7 to 21.4	1.8
70.7 to 79.2	21.5 to 26.2	1.6
79.3 to 90.5	26.3 to 32.5	1.4

§ 141.12 Maximum contaminant levels for organic chemicals.

The following are the maximum contaminant levels for organic chemicals. They apply only to community water systems. Compliance with maximum contaminant levels for organic chemicals is calculated pursuant to § 141.24.

	Level, milligrams per liter
(a) Chlorinated hydrocarbons:	
Endrin (1,2,3,4,10, 10-hexachloro-6,7-epoxy-1,4, 4a,5,6,7,8,8a-octahydro-1,4-endo, endo-5,8 - dimethano naphthalene).	0.0002
Lindane (1,2,3,4,5,6-hexachlorocyclohexane, gamma isomer).	0.004
Methoxychlor (1,1,1-Trichloro-2, 2 - bis [p-methoxyphenyl] ethane).	0.1
Toxaphene ($C_{10}H_{10}Cl_8$-Technical chlorinated camphene, 67-69 percent chlorine).	0.005

(b) Chlorophenoxys:	
2,4-D, (2,4-Dichlorophenoxyacetic acid).	0.1
2,4,5-TP Silvex (2,4,5-Trichlorophenoxypropionic acid).	0.01

§ 141.13 Maximum contaminant levels for turbidity.

The maximum contaminant levels for turbidity are applicable to both community water systems and non-community water systems using surface water sources in whole or in part. The maximum contaminant levels for turbidity in drinking water, measured at a representative entry point(s) to the distribution system, are:

(a) One turbidity unit (TU), as determined by a monthly average pursuant to § 141.22, except that five or fewer turbidity units may be allowed if the supplier of water can demonstrate to the State that the higher turbidity does not do any of the following:

(1) Interfere with disinfection;

(2) Prevent maintenance of an effective disinfectant agent throughout the distribution system; or

(3) Interfere with microbiological determinations.

(b) Five turbidity units based on an average for two consecutive days pursuant to § 141.22.

§ 141.14 Maximum microbiological contaminant levels.

The maximum contaminant levels for coliform bacteria, applicable to community water systems and non-community water systems, are as follows:

(a) When the membrane filter technique pursuant to § 141.21(a) is used, the number of coliform bacteria shall not exceed any of the following:

(1) One per 100 milliliters as the arithmetic mean of all samples examined per month pursuant to § 141.21 (b) or (c);

(2) Four per 100 milliliters in more than one sample when less than 20 are examined per month; or

(3) Four per 100 milliliters in more than five percent of the samples when 20 or more are examined per month.

(b) (1) When the fermentation tube method and 10 milliliter standard portions pursuant to § 141.21(a) are used, coliform bacteria shall not be present in any of the following:

(i) more than 10 percent of the portions in any month pursuant to § 141.21 (b) or (c);

(ii) three or more portions in more than one sample when less than 20 samples are examined per month; or

(iii) three or more portions in more than five percent of the samples when 20 or more samples are examined per month.

(2) When the fermentation tube method and 100 milliliter standard portions pursuant to § 141.21(a) are used, coliform bacteria shall not be present in any of the following:

(i) more than 60 percent of the portions in any month pursuant to § 141.21 (b) or (c);

(ii) five portions in more than one sample when less than five samples are examined per month; or

(iii) five portions in more than 20 percent of the samples when five or more samples are examined per month.

(c) For community or non-community systems that are required to sample at a rate of less than 4 per month, compliance with paragraphs (a), (b)(1), or (b)(2) of this section shall be based upon sampling during a 3 month period, except that, at the discretion of the State, compliance may be based upon sampling during a one-month period.

Subpart C—Monitoring and Analytical Requirements

§ 141.21 Microbiological contaminant sampling and analytical requirements.

(a) Suppliers of water for community water systems and non-community water systems shall analyze for coliform bacteria for the purpose of determining compliance with § 141.14. Analyses shall be conducted in accordance with the analytical recommendations set forth in "Standard Methods for the Examination of Water and Wastewater," American Public Health Association, 13th Edition, pp. 662–688, except that a standard sample size shall be employed. The standard sample used in the membrane filter procedure shall be 100 milliliters. The standard sample used in the 5 tube most probable number (MPN) procedure (fermentation tube method) shall be 5 times the standard portion. The standard portion is either 10 milliliters or 100 milliliters as described in § 141.14 (b) and (c). The samples shall be taken at points which are representative of the conditions within the distribution system.

(b) The supplier of water for a community water system shall take coliform density samples at regular time intervals, and in number proportionate to the population served by the system. In no event shall the frequency be less than as set forth below:

Population served:	*Minimum number of samples per month*
25 to 1,000	1
1,001 to 2,500	2
2,501 to 3,300	3
3,301 to 4,100	4
4,101 to 4,900	5
4,901 to 5,800	6
5,801 to 6,700	7
6,701 to 7,600	8
7,601 to 8,500	9
8,501 to 9,400	10
9,401 to 10,300	11
10,301 to 11,100	12
11,101 to 12,000	13
12,001 to 12,900	14
12,901 to 13,700	15
13,701 to 14,600	16
14,601 to 15,500	17
15,501 to 16,300	18
16,301 to 17,200	19
17,201 to 18,100	20
18,101 to 18,900	21
18,901 to 19,800	22
19,801 to 20,700	23
20,701 to 21,500	24
21,501 to 22,300	25
22,301 to 23,200	26
23,201 to 24,000	27
24,001 to 24,900	28
24,901 to 25,000	29
25,001 to 28,000	30
28,001 to 33,000	35
33,001 to 37,000	40
37,001 to 41,000	45
41,001 to 46,000	50
46,001 to 50,000	55
50,001 to 54,000	60
54,001 to 59,000	65
59,001 to 64,000	70
64,001 to 70,000	75
70,001 to 76,000	80
76,001 to 83,000	85
83,001 to 90,000	90
90,001 to 96,000	95
96,001 to 111,000	100
111,001 to 130,000	110
130,001 to 160,000	120
160,001 to 190,000	130
190,001 to 220,000	140
220,001 to 250,000	150
250,001 to 290,000	160
290,001 to 320,000	170
320,001 to 360,000	180
360,001 to 410,000	190
410,001 to 450,000	200
450,001 to 500,000	210
500,001 to 550,000	220
550,001 to 600,000	230
600,001 to 660,000	240
660,001 to 720,000	250
720,001 to 780,000	260
780,001 to 840,000	270
840,001 to 910,000	280
910,001 to 970,000	290
970,001 to 1,050,000	300
1,050,001 to 1,140,000	310
1,140,001 to 1,230,000	320
1,230,001 to 1,320,000	330
1,320,001 to 1,420,000	340
1,420,001 to 1,520,000	350
1,520,001 to 1,630,000	360
1,630,001 to 1,730,000	370
1,730,001 to 1,850,000	380
1,850,001 to 1,970,000	390
1,970,001 to 2,060,000	400
2,060,001 to 2,270,000	410
2,270,001 to 2,510,000	420
2,510,001 to 2,750,000	430
2,750,001 to 3,020,000	440
3,020,001 to 3,320,000	450
3,320,001 to 3,620,000	460
3,620,001 to 3,960,000	470
3,960,001 to 4,310,000	480
4,310,001 to 4,690,000	490
4,690,001 or more	500

Based on a history of no coliform bacterial contamination and on a sanitary survey by the State showing the water system to be supplied solely by a protected ground water source and free of sanitary defects, a community water system serving 25 to 1,000 persons, with written permission from the State, may reduce this sampling frequency except that in no case shall it be reduced to less than one per quarter.

(c) The supplier of water for a non-community water system shall sample for coliform bacteria in each calendar quarter during which the system provides water to the public. Such sampling shall begin within two years after the effective date of this part. If the State, on the basis of a sanitary survey, determines that some other frequency is more appropriate, that frequency shall be the frequency required under these regulations. Such frequency shall be confirmed or changed on the basis of subsequent surveys.

(d) (1) When the coliform bacteria in a single sample exceed four per 100 milliliters (§ 141.14(a)), at least two consecutive daily check samples shall be collected and examined from the same sampling point. Additional check samples shall be collected daily, or at a frequency estab-

lished by the State, until the results obtained from at least two consecutive check samples show less than one coliform bacterium per 100 milliliters.

(2) When coliform bacteria occur in three or more 10 ml portions of a single sample (§ 141.14(b)(1)), at least two consecutive daily check samples shall be collected and examined from the same sampling point. Additional check samples shall be collected daily, or at a frequency established by the State, until the results obtained from at least two consecutive check samples show no positive tubes.

(3) When coliform bacteria occur in all five of the 100 ml portions of a single sample (§ 141.14(b)(2)), at least two daily check samples shall be collected and examined from the same sampling point. Additional check samples shall be collected daily, or at a frequency established by the State, until the results obtained from at least two consecutive check samples show no positive tubes.

(4) The location at which the check samples were taken pursuant to paragraphs (d) (1), (2), or (3) of this section shall not be eliminated from future sampling without approval of the State. The results from all coliform bacterial analyses performed pursuant to this subpart, except those obtained from check samples and special purpose samples, shall be used to determine compliance with the maximum contaminant level for coliform bacteria as established in § 141.14. Check samples shall not be included in calculating the total number of samples taken each month to determine compliance with § 141.21 (b) or (c).

(e) When the presence of coliform bacteria in water taken from a particular sampling point has been confirmed by any check samples examined as directed in paragraphs (d) (1), (2), or (3) of this section, the supplier of water shall report to the State within 48 hours.

(f) When a maximum contaminant level set forth in paragraphs (a), (b) or (c) of § 141.14 is exceeded, the supplier of water shall report to the State and notify the public as prescribed in § 141.31 and § 141.32.

(g) Special purpose samples, such as those taken to determine whether disinfection practices following pipe placement, replacement, or repair have been sufficient, shall not be used to determine compliance with § 141.14 or § 141.21 (b) or (c).

(h) A supplier of water of a community water system or a non-community water system may, with the approval of the State and based upon a sanitary survey, substitute the use of chlorine residual monitoring for not more than 75 percent of the samples required to be taken by paragraph (b) of this section, *Provided*, That the supplier of water takes chlorine residual samples at points which are representative of the conditions within the distribution system at the frequency of at least four for each substituted microbiological sample. There shall be at least daily determinations of chlorine residual. When the supplier of water exercises the option provided in this paragraph (h) of this section, he shall maintain no less than 0.2 mg/l free chlorine throughout the public water distribution system. When a particular sampling point has been shown to have a free chlorine residual less than 0.2 mg/l, the water at that location shall be retested as soon as practicable and in any event within one hour. If the original analysis is confirmed, this fact shall be reported to the State within 48 hours. Also, if the analysis is confirmed, a sample for coliform bacterial analysis must be collected from that sampling point as soon as practicable and preferably within one hour, and the results of such analysis reported to the State within 48 hours after the results are known to the supplier of water. Analyses for residual chlorine shall be made in accordance with "Standard Methods for the Examination of Water and Wastewater," 13th Ed., pp. 129–132. Compliance with the maximum contaminant levels for coliform bacteria shall be determined on the monthly mean or quarterly mean basis specified in § 141.14, including those samples taken as a result of failure to maintain the required chlorine residual level. The State may withdraw its approval of the use of chlorine residual substitution at any time.

§ 141.22 Turbidity sampling and analytical requirements.

(a) Samples shall be taken by suppliers of water for both community water systems and non-community water systems at a representative entry point(s) to the water distribution system at least once per day, for the purpose of making turbidity measurements to determine compliance with § 141.13. The measurement shall be made by the Nephelometric Method in accordance with the recommendations set forth in "Standard Methods for the Examination of Water and Wastewater," American Public Health Association, 13th Edition, pp. 350–353, or "Methods for Chemical Analysis of Water and Wastes," pp. 295–298, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(b) If the result of a turbidity analysis indicates that the maximum allowable limit has been exceeded, the sampling and measurement shall be confirmed by resampling as soon as practicable and preferably within one hour. If the repeat sample confirms that the maximum allowable limit has been exceeded, the supplier of water shall report to the State within 48 hours. The repeat sample shall be the sample used for the purpose of calculating the monthly average. If the monthly average of the daily samples exceeds the maximum allowable limit, or if the average of two samples taken on consecutive days exceeds 5 TU, the supplier of water shall report to the State and notify the public as directed in § 141.31 and § 141.32.

(c) Sampling for non-community water systems shall begin within two years after the effective date of this part.

(d) The requirements of this § 141.22 shall apply only to public water systems which use water obtained in whole or in part from surface sources.

§ 141.23 Inorganic chemical sampling and analytical requirements.

(a) Analyses for the purpose of determining compliance with § 141.11 are required as follows:

(1) Analyses for all community water systems utilizing surface water sources shall be completed within one year following the effective date of this part. These analyses shall be repeated at yearly intervals.

(2) Analyses for all community water systems utilizing only ground water sources shall be completed within two years following the effective date of this part. These analyses shall be repeated at three-year intervals.

(3) For non-community water systems, whether supplied by surface or ground water sources, analyses for nitrate shall be completed within two years following the effective date of this part. These analyses shall be repeated at intervals determined by the State.

(b) If the result of an analysis made pursuant to paragraph (a) indicates that the level of any contaminant listed in § 141.11 exceeds the maximum contaminant level, the supplier of water shall report to the State within 7 days and initiate three additional analyses at the same sampling point within one month.

(c) When the average of four analyses made pursuant to paragraph (b) of this section, rounded to the same number of significant figures as the maximum contaminant level for the substance in question, exceeds the maximum contaminant level, the supplier of water shall notify the State pursuant to § 141.31 and give notice to the public pursuant to § 141.32. Monitoring after public notification shall be at a frequency designated by the State and shall continue until the maximum contaminant level has not been exceeded in two successive samples or until a monitoring schedule as a condition to a variance, exemption or enforcement action shall become effective.

(d) The provisions of paragraphs (b) and (c) of this section notwithstanding, compliance with the maximum contaminant level for nitrate shall be determined on the basis of the mean of two analyses. When a level exceeding the maximum contaminant level for nitrate is found, a second analysis shall be initiated within 24 hours, and if the mean of the two analyses exceeds the maximum contaminant level, the supplier of water shall report his findings to the State pursuant to § 141.31 and shall notify the public pursuant to § 141.32.

(e) For the initial analyses required by paragraph (a)(1), (2) or (3) of this section, data for surface waters acquired within one year prior to the effective date and data for ground waters acquired within 3 years prior to the effective date of this part may be substituted at the discretion of the State.

(f) Analyses conducted to determine compliance with § 141.11 shall be made in accordance with the following methods:

(1) Arsenic—Atomic Absorption Method, "Methods for Chemical Analysis of Water and Wastes," pp. 95–96, Environ-

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mental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(2) Barium—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 210–215, or "Methods for Chemical Analysis of Water and Wastes," pp. 97–98, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(3) Cadmium—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 210–215, or "Methods for Chemical Analysis of Water and Wastes," pp. 101–103, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(4) Chromium—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 210–215, or "Methods for Chemical Analysis of Water and Wastes," pp. 105–106 Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(5) Lead—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 210–215, or "Methods for Chemical Analysis of Water and Wastes," pp. 112–113, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(6) Mercury—Flameless Atomic Absorption Method, "Methods for Chemical Analysis of Water and Wastes," pp. 118–126, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(7) Nitrate—Brucine Colorimetric Method, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 461–464, or Cadmium Reduction Method, "Methods for Chemical Analysis of Water and Wastes," pp. 201–206, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(8) Selenium—Atomic Absorption Method, "Methods for Chemical Analysis of Water and Wastes," p. 145, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(9) Silver—Atomic Absorption Method, "Standard Methods for the Examination of Water and Wastewater", 13th Edition, pp. 210–215, or "Methods for Chemical Analysis of Water and Wastes", p. 146, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

(10) Fluoride—Electrode Method, "Standard Methods for the Examination of Water and Wastewater", 13th Edition, pp. 172–174, or "Methods for Chemical Analysis of Water and Wastes," pp. 65–67, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974, or Colorimetric Method with Preliminary Distillation, "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 171–172 and 174–176, or "Methods for Chemical Analysis of Water and Wastes," pp. 59–60, Environmental Protection Agency, Office of Technology Transfer, Washington, D.C. 20460, 1974.

§ 141.24 Organic chemical sampling and analytical requirements.

(a) An analysis of substances for the purpose of determining compliance with § 141.12 shall be made as follows:

(1) For all community water systems utilizing surface water sources, analyses shall be completed within one year following the effective date of this part. Samples analyzed shall be collected during the period of the year designated by the State as the period when contamination by pesticides is most likely to occur. These analyses shall be repeated at intervals specified by the State but in no event less frequently than at three year intervals.

(2) For community water systems utilizing only ground water sources, analyses shall be completed by those systems specified by the State.

(b) If the result of an analysis made pursuant to paragraph (a) of this section indicates that the level of any contaminant listed in § 141.12 exceeds the maximum contaminant level, the supplier of water shall report to the State within 7 days and initiate three additional analyses within one month.

(c) When the average of four analyses made pursuant to paragraph (b) of this section, rounded to the same number of significant figures as the maximum contaminant level for the substance in question, exceeds the maximum contaminant level, the supplier of water shall report to the State pursuant to § 141.31 and give notice to the public pursuant to § 141.32. Monitoring after public notification shall be at a frequency designated by the State and shall continue until the maximum contaminant level has not been exceeded in two successive samples or until a monitoring schedule as a condition to a variance, exemption or enforcement action shall become effective.

(d) For the initial analysis required by paragraph (a) (1) and (2) of this section, data for surface water acquired within one year prior to the effective date of this part and data for ground water acquired within three years prior to the effective date of this part may be substituted at the discretion of the State.

(e) Analyses made to determine compliance with § 141.12(a) shall be made in accordance with "Method for Organochlorine Pesticides in Industrial Effluents," MDQARL, Environmental Protection Agency, Cincinnati, Ohio, November 28, 1973.

(f) Analyses made to determine compliance with § 141.12(b) shall be conducted in accordance with "Methods for Chlorinated Phenoxy Acid Herbicides in Industrial Effluents," MDQARL, Environmental Protection Agency, Cincinnati, Ohio, November 28, 1973.

§ 141.27 Alternative analytical techniques.

With the written permission of the State, concurred in by the Administrator of the U.S. Environmental Protection Agency, an alternative analytical technique may be employed. An alternative technique shall be acceptable only if it is substantially equivalent to the prescribed test in both precision and accuracy as it relates to the determination of compliance with any maximum contaminant level. The use of the alternative analytical technique shall not decrease the frequency of monitoring required by this part.

§ 141.28 Approved laboratories.

For the purpose of determining compliance with § 141.21 through § 141.27, samples may be considered only if they have been analyzed by a laboratory approved by the State except that measurements for turbidity and free chlorine residual may be performed by any person acceptable to the State.

§ 141.29 Monitoring of consecutive public water systems.

When a public water system supplies water to one or more other public water systems, the State may modify the monitoring requirements imposed by this part to the extent that the interconnection of the sysems jusifies treating them as a single system for monitoring purposes. Any modified monitoring shall be conducted pursuant to a schedule specified by the State and concurred in by the Administrator of the U.S. Environmental Protection Agency.

Subpart D—Reporting, Public Notification and Record Keeping

§ 141.31 Reporting requirements.

(a) Except where a shorter reporting period is specified in this part, the supplier of water shall report to the State within 40 days following a test, measurement or analysis required to be made by this part, the results of that test, measurement or analysis.

(b) The supplier of water shall report to the State within 48 hours the failure to comply with any primary drinking water regulation (including failure to comply with monitoring requirements) set forth in this part.

(c) The supplier of water is not required to report analytical results to the State in cases where a State laboratory performs the analysis and reports the results to the State office which would normally receive such notification from the supplier.

§ 141.32 Public notification.

(a) If a community water system fails to comply with an applicable maximum contaminant level established in Subpart B, fails to comply with an applicable testing procedure established in Subpart C of this part, is granted a variance or an exemption from an applicable maximum contaminant level, fails to comply with the requirements of any schedule prescribed pursuant to a variance or exemption, or fails to perform any monitoring required pursuant to Section 1445 (a) of the Act, the supplier of water shall notify persons served by the system of the failure or grant by inclusion of a notice in the first set of water bills of the system issued after the failure or grant

and in any event by written notice within three months. Such notice shall be repeated at least once every three months so long as the system's failure continues or the variance or exemption remains in effect. If the system issues water bills less frequently than quarterly, or does not issue water bills, the notice shall be made by or supplemented by another form of direct mail.

(b) If a community water system has failed to comply with an applicable maximum contaminant level, the supplier of water shall notify the public of such failure, in addition to the notification required by paragraph (a) of this section, as follows:

(1) By publication on not less than three consecutive days in a newspaper or newspapers of general circulation in the area served by the system. Such notice shall be completed within fourteen days after the supplier of water learns of the failure.

(2) By furnishing a copy of the notice to the radio and television stations serving the area served by the system. Such notice shall be furnished within seven days after the supplier of water learns of the failure.

(c) If the area served by a community water system is not served by a daily newspaper of general circulation, notification by newspaper required by paragraph (b) of this section shall instead be given by publication on three consecutive weeks in a weekly newspaper of general circulation serving the area. If no weekly or daily newspaper of general circulation serves the area, notice shall be given by posting the notice in post offices within the area served by the system.

(d) If a non-community water system fails to comply with an applicable maximum contaminant level established in Subpart B of this part fails to comply with an applicable testing procedure established in Subpart C of this part, is granted a variance or an exemption from an applicable maximum contaminant level, fails to comply with the requirement of any schedule prescribed pursuant to a variance or exemption or fails to perform any monitoring required pursuant to Section 1445(a) of the Act, the supplier of water shall given notice of such failure or grant to the persons served by the system. The form and manner of such notice shall be prescribed by the State, and shall insure that the public using the system is adequately informed of the failure or grant.

(e) Notices given pursuant to this section shall be written in a manner reasonably designed to inform fully the users of the system. The notice shall be conspicuous and shall not use unduly technical language, unduly small print or other methods which would frustrate the purpose of the notice. The notice shall disclose all material facts regarding the subject including the nature of the problem and, when appropriate, a clear statement that a primary drinking water regulation has been violated and any preventive measures that should be taken by the public. Where appropriate, or where designated by the State, bilingual notice shall be given. Notices may include a balanced explanation of the significance or seriousness to the public health of the subject of the notice, a fair explanation of steps taken by the system to correct any problem and the results of any additional sampling.

(f) Notice to the public required by this section may be given by the State on behalf of the supplier of water.

(g) In any instance in which notification by mail is required by paragraph (a) of this section but notification by newspaper or to radio or television stations is not required by paragraph (b) of this section, the State may order the supplier of water to provide notification by newspaper and to radio and television stations when circumstances make more immediate or broader notice appropriate to protect the public health.

§ 141.33 Record maintenance.

Any owner or operator of a public water system subject to the provisions of this part shall retain on its premises or at a convenient location near its premises the following records:

(a) Records of bacteriological analyses made pursuant to this part shall be kept for not less than 5 years. Records of chemical analyses made pursuant to this part shall be kept for not less than 10 years. Actual laboratory reports may be kept, or data may be transferred to tabular summaries, provided that the following information is included:

(1) The date, place, and time of sampling, and the name of the person who collected the sample;

(2) Identification of the sample as to whether it was a routine distribution system sample, check sample, raw or process water sample or other special purpose sample;

(3) Date of analysis;

(4) Laboratory and person responsible for performing analysis;

(5) The analytical technique/method used; and

(6) The results of the analysis.

(b) Records of action taken by the system to correct violations of primary drinking water regulations shall be kept for a period not less than 3 years after the last action taken with respect to the particular violation involved.

(c) Copies of any written reports, summaries or communications relating to sanitary surveys of the system conducted by the system itself, by a private consultant, or by any local, State or Federal agency, shall be kept for a period not less than 10 years after completion of the sanitary survey involved.

(d) Records concerning a variance or exemption granted to the system shall be kept for a period ending not less than 5 years following the expiration of such variance or exemption.

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FROM p. 57332:

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 141

[FRL 1535-7]

Interim Primary Drinking Water Regulations; Amendments

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

EFFECTIVE DATE: These amendments to the regulations will be effective August 27, 1980 except that sodium monitoring and reporting, determination of the types of materials in distribution systems, and monitoring and reporting corrosivity characteristics will be effective 18 months following the date of promulgation. The sodium and corrosion requirements must be completed within 12 months following the effective date.

P 57343:

4. Amending § 141.14 (a)(1), (b)(1)(i), (b)(2)(i), and revising (d) to read as follows:

§ 141.14 Maximum microbiological contaminant levels.

(a) * * *

(1) One per 100 milliliters as the arithmetic mean of all samples examined per compliance period pursuant to § 141.21(b) or (c), except that, at the primacy Agency's discretion systems required to take 10 or fewer samples per month may be authorized to exclude one positive routine sample per month from the monthly calculation if: (i) as approved on a case-by-case basis the State determines and indicates in writing to the public water system that no unreasonable risk to health existed under the conditions of this modification. This determination should be based upon a number of factors not limited to the following: (A) the system provided and had maintained an active disinfectant residual in the distribution system, (B) the potential for contamination as indicated by a sanitary survey, and (C) the history of the water quality at the public water system (e.g. MCL or monitoring violations); (ii) the supplier initiates a check sample on each of two consecutive days from the same sampling point within 24 hours after notification that the routine sample is positive, and each of these check samples is negative; and (iii) the original positive routine sample is reported and recorded by the supplier pursuant to § 141.31(a) and § 141.33(a). The supplier shall report to the State its compliance with the conditions specified in this paragraph and a summary of the corrective action taken to resolve the prior positive sample result. If a positive routine sample is not used for the monthly calculation, another routine sample must be analyzed for compliance purposes. This provision may be used only once during two consecutive compliance periods.

(b)(1) * * *

(i) More than 10 percent of the portions (tubes) in any one month pursuant to § 141.21 (b) or (c) except that, at the State's discretion, systems required to take 10 or fewer samples per month may be authorized to exclude one positive routine sample resulting in one or more positive tubes per month from the monthly calculation if: (A) as approved on a case-by-case basis the State determines and indicates in writing to the public water system that no unreasonable risk to health existed under the conditions of this modification. This determination should be based upon a number of factors not limited to the following: (*1*) the system provided and had maintained an active disinfectant residual in the distribution system, (*2*) the potential for contamination as indicated by a sanitary survey, and (*3*) the history of the water quality at the public water system (e.g. MCL or monitoring violations); (B) the supplier initiates a check sample on each of two consecutive days from the sampling point within 24 hours after notification that the routine sample is positive, and each of these check samples is negative; and (C) the original positive routine sample is reported and recorded by the supplier pursuant to § 141.31(a) and § 141.33(a). The supplier shall report to the State its compliance with the conditions specified in this paragraph and report the action taken to resolve the prior positive sample result. If a positive routine sample is not used for the monthly calculation, another routine sample must be analyzed for compliance purposes. This provision may be used only once during two consecutive compliance periods.

(b)(2) * * *

(i) More than 60 percent of the portions (tubes) in any month pursuant to § 141.21 (b) or (c), except that, State discretion, systems required to take 10 or fewer samples per month may be authorized to exclude one positive routine sample resulting in one or more positive tubes per month from the monthly calculation if: (A) as approved on a case-by-case basis the State determines and indicates in writing to the public water system that no unreasonable risk to health existed under the conditions of this modification. This determination should be based upon a number of factors not limited to the following: (*1*) the system provided and had maintained an active disinfectant residual in the distribution system, (*2*) the potential for contamination as indicated by a sanitary survey, and (iii) the history of the water quality at the public water system (e.g. MCL or monitoring violations); (B) the supplier initiates two consecutive daily check samples from the same sampling point within 24 hours after notification that the routine sample is positive, and each of these check samples is negative; and (C) the original positive routine sample is reported and recorded by the supplier pursuant to § 141.31(a) and § 141.33(a). The supplier shall report to the State its compliance with the conditions specified in this paragraph and a summary of the corrective action taken to resolve the prior positive sample result. If a positive routine sample is not used for the monthly calculation, another routine sample must be analyzed for compliance purposes. This provision may be used only once during two consecutive compliance periods.

* * * * *

(d) If an average MCL violation is caused by a single sample MCL violation, then the case shall be treated as one violation with respect to the public notification requirements of § 141.32.

8. Amending § 141.21 (a) and (c) to read as follows and adding (i):

§ 141.21 Microbiological contaminant sampling and analytical requirements.

(a) Suppliers of water for community and non-community water systems shall analyze or use the services of an approved laboratory for coliform bacteria to determine compliance with § 141.14. Analyses shall be conducted in accordance with the analytical recommendations set forth in "Standard Methods for the Examination of Water and Wastewater," American Public Health Association, 14th Edition, Method 908A, Paragraphs 1, 2 and 3—pp. 916–918; Method 908D, Table 908: I—p. 923; Method 909A, pp. 928–935, or "Microbiological Methods for Monitoring the Environment, Water and Wastes," U.S. EPA, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268—EPA–600/8–78–017, December 1978. Available from ORD Publications, CERI, U.S. EPA, Cincinnati, Ohio 45268. Part III, Section

B 1.0 through 2.6.2, pp. 108–112; 2.7 through 2.7.2(c), pp. 112–113; Part III, Section B 4.0 through 4.6.4(c), pp. 114–118, except that a standard sample size shall be employed. The standard sample used in the membrane filter procedure shall be 100 milliliters. The standard sample used in the 5 tube most probable number (MPN) procedure (fermentation tube method) shall be 5 times the standard portion. The standard portion is either 10 milliliters or 100 milliliters as described in § 141.14 (b) and (c). The samples shall be taken at points which are representative of the conditions within the distribution system.

* * * * *

(c) The supplier of water for a non-community water system shall be responsible for sampling coliform bacteria in each calendar quarter that the system provides water to the public. Such sampling shall begin within two years after promulgation. The State can adjust the monitoring frequency on the basis of a sanitary survey, the existence of additional safeguards such as a protective and enforced well code, or accumulated analytical data. Such frequency shall be confirmed or modified on the basis of subsequent surveys or data. The frequency shall not be reduced until the non-community water system has performed at least one coliform analysis of its drinking water and shown to be in compliance with § 141.14.

* * * * *

(i) The State has the authority to determine compliance or initiate enforcement action based upon analytical results or other information compiled by their sanctioned representatives and agencies.

9. Amending § 141.22(a) to read as follows and adding (e):

§ 141.22 Turbidity sampling and analytical requirements.

(a) Samples shall be taken by suppliers of water for both community and non-community water systems at a representative entry point(s) to the water distribution system at least once per day, for the purpose of making turbidity measurements to determine compliance with § 141.13. If the State determines that a reduced sampling frequency in a non-community system will not pose a risk to public health, it can reduce the required sampling frequency. The option of reducing the turbidity frequency shall be permitted only in those public water systems that practice disinfection and which maintain an active residual disinfectant in the distribution system, and in those cases where the State has indicated in writing that no unreasonable risk to health existed under the circumstances of this option. The turbidity measurements shall be made by the Nephelometric Method in accordance with the recommendations set forth in "Standard Methods for Examination of Water and Wastewater," American Public Health Association, 14th Edition, pp. 132–134; or Method 180.1,1-Nephrometric Method.

* * * * *

(e) The State has the authority to determine compliance or initiate enforcement action based upon analytical results or other information compiled by their sanctioned representatives and agencies.

10. Amending § 141.23(a)(3), adding (a)(4) and amending (f) (1) through (10) to read as follows:

§ 141.23 Inorganic chemical sampling and analytical requirements.

(a) * * *

(3) For non-community water systems, whether supplied by surface or ground sources, analyses for nitrate shall be completed by December 24, 1980. These analyses shall be repeated at intervals determined by the State.

(4) The State has the authority to determine compliance or initiate enforcement action based upon analytical results and other information compiled by their sanctioned representatives and agencies.

* * * * *

(f) * * *

(1) Arsenic—Method [1] 206.2, Atomic Absorption Furnace Technique; or Method [1] 206.3, or Method [4] D2972–78A, or Method [2] 301.A VII, pp. 159–162, or Method [3] I–1062–78, pp. 61–63, Atomic Absorption—Gaseous Hydride; or Method [1] 206.4, or Method [4] D–2972–78A, or Method [2] 404–A and 404–B(4), Spectrophotometric, Silver Diethyldithiocarbamate.

(2) Barium—Method [1] 208.1, or Method [2] 301–A IV, pp. 152–155, Atomic Absorption—Direct Aspiration; or Method [1] 208.2, Atomic Absorption Furnace Technique.

(3) Cadmium—Method [1] 213.1, or Method [4] 3557–78A or B, or Method [2] 301–A II or III, pp. 148–152, Atomic Absorption—Direct Aspiration; or Method [1] 213.2, Atomic Absorption Furnace Technique.

(4) Chromium—Method [1] 218.1, or Method [4] D–1687–77D, or Method [2] 301–A II or III, pp. 148–152, Atomic Absorption—Direct Aspiration; or Chromium—Method [1] 218.2, Atomic Absorption Furnace Technique.

(5) Lead—Method [1] 239.1, or Method [4] D–3559–78A or B, or Method [2] 301–A II or III, pp. 148–152, Atomic Absorption—Direct Aspiration; or Method [1] 239.2, Atomic Absorption Furnace Technique.

(6) Mercury—Method [1] 245.1, or Method [4] D–3223–79, or Method [2] 301–A VI, pp. 158–159, Manual Cold Vapor Technique; or Method [1] 245.2, Automated Cold Vapor Technique.

(7) Nitrate—Method [1] 352.1, or Method [4] D–992–71, or Method [2] 419–D, pp. 427–429, Colorimetric Brucine; or Method [1] 353.3, or Method [4] D–3867–79B, or Method [2] 419–C, pp. 423–427, Spectrometric, Cadmium Reduction; Method [1] 353.1, Automated Hydrazine Reduction; or Method [1] 353.2, or Method [4] D–3867–79A, or Method [2] 605, pp. 620–624, Automated Cadmium Reduction.

(8) Selenium—Method [1] 270.2, Atomic Absorption Technique; or Method [1] 270.3; or Method [3] I–1667–78, pp. 237–239, or Method [4] D–3859–79, or Method [2] 301–A VII, pp. 159–162, Hydride Generation—Atomic Absorption Spectrophotometry.

(9) Silver—Method [1] 272.1, or Method [2] 301–A II, Atomic Absorption—Direct Aspiration; or Method [1] 272.2, Atomic Absorption Techniques Furnace Technique.

(10) Fluoride—Electrode Method, or SPADNS Method, Method [2] 414–B and C, pp. 391–394, or Method [1] 340.1, "Colorimetric SPADNS with Bellack Distillation," or Method [1] 340.2, "Potentimetric Ion Selective Electrode;" or ASTM Method [4] D1179–72; or Colorimetric Method with Preliminary Distillation, Method [2] 603, Automated Complexone Method (Alizarin Fluoride Blue) pp. 614–616; or Automated Electrode Method, "Fluoride in Water and Wastewater," Industrial Method #380–75WE, Technicon Industrial Systems, Tarrytown, New York 10591, February 1976, or "Fluoride in Water

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[1] "Methods of Chemical Analysis of Water and Wastes," EPA Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268 (EPA–600/4–79–020), March 1979. Available from ORD Publications, CERI, EPA, Cincinnati, Ohio 45268. For approved analytical procedures for metals, the technique applicable to total metals must be used.

[2] "Standard Methods for the Examination of Water and Wastewater," 14th Edition, American Public Health Association, American Water Works Association, Water Pollution Control Federation, 1976.

[3] Techniques of Water—Resources Investigation of the United States Geological Survey, Chapter A–1, "Methods for Determination of Inorganic Substances in Water and Fluvial Sediments," Book 5, 1979, Stock #024–001–03177–9. Available from Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.

[4] Annual Book of ASTM Standards, part 31 Water, American Society for Testing and Materials, 1976, Race Street, Philadelphia, Pennsylvania 19103.

and Wastewater Industrial Method #129-71W," Technicon Industrial Systems, Tarrytown, New York 10591, December 1972; or Fluoride, Total, Colorimetric, Zirconium—Eriochrome Cyanine R Method [3]—I-3325-78, pp. 365-367.

11. Amending §141.24(a)(3), (e) and (f) to read as follows:

§ 141.24 Organic chemical sampling and analytical requirements.

(a) • • •

(3) The State has the authority to determine compliance or initiate enforcement action based upon analytical results and other information compiled by their sanctioned representatives and agencies.

• • • • •

(e) Analysis made to determine compliance with § 141.12(a) shall be made in accordance with "Methods for Organochlorine Pesticides and Chlorophenoxy Acid Herbicides in Drinking Water and Raw Source Water," available from ORD Publications, CERI, EPA, Cincinnati, Ohio 45268; or "Organochlorine Pesticides in Water," 1977 Annual Book of ASTM Standards, part 31, Water, Method D3088; or Method 509-A, pp. 555-565; [3] or Gas Chromatographic Methods for Analysis of Organic Substances in Water,[4] USGS, Book 5, Chapter A-5, pp. 24-39.

(f) Analysis made to determine compliance with § 141.12(b) shall be conducted in accordance with "Methods for Organochlorine Pesticides and Chlorophenoxy Acid Herbicides in Drinking Water and Raw Source Water," available from ORD Publications, CERI, EPA, Cincinnati, Ohio 45268; or "Chlorinated Phenoxy Acid Herbicides in Water," 1977 Annual Book of ASTM Standards, part 31, Method D3478; or Method 509-B, pp. 555-5692; [3] or Gas Chromatographic Methods for Analysis of Organic Substances in Water,[4] USGS, Book 5, Chapter A-3, pp. 24-39.

§ 141.25 [Amended]

12. Amending § 141.25 to add (e):

(e) The State has the authority to determine compliance or initiate enforcement action based upon analytical results or other information compiled by their sanctioned representatives and agencies.

13. Amending § 141.27(a) to read as follows:

§ 141.27 Alternate analytical techniques.

(a) With the written permission of the State, concurred in by the Administrator of the U.S. EPA, an alternate analytical technique may be employed. An alternate technique shall be accepted only if it is substantially equivalent to the prescribed test in both precision and accuracy as it relates to the determination of compliance with any MCL. The use of the alternate analytical technique shall not decrease the frequency of monitoring required by this part.

14. Amending § 141.28 to read as follows:

§ 141.28 Approved laboratories.

(a) For the purpose of determining compliance with § 141.21 through § 141.27, samples may be considered only if they have been analyzed by a laboratory approved by the State except that measurements for turbidity, free chlorine residual, temperature and pH may be performed by any person acceptable to the State.

(b) Nothing in this Part shall be construed to preclude the State or any duly designated representative of the State from taking samples or from using the results from such samples to determine compliance by a supplier of water with the applicable requirements of this Part.

15. Amending § 141.31 (a) and (c) and adding paragraphs (d) and (e) to read as follows:

§ 141.31 Reporting requirements.

(a) Except where a shorter period is specified in this part, the supplier of water shall report to the State the results of any test measurement or analysis required by this part within (A) the first ten days following the month in which the result is received or (B) the first ten days following the end of the required monitoring period as stipulated by the State, whichever of these is shortest.

• • • • •

(d) The water supply system, within ten days of completion of each public notification required pursuant to § 141.32, shall submit to the State a representative copy of each type of notice distributed, published, posted, and/or made available to the persons served by the system and/or to the media.

(e) The water supply system shall submit to the State within the time stated in the request copies of any records required to be maintained under § 141.33 hereof or copies of any documents then in existence which the State or the Administrator is entitled to inspect pursuant to the authority of § 1445 of the Safe Drinking Water Act or the equivalent provisions of State law.

16. Amending § 141.32 (b)(3) and (d) to read as follows:

§ 141.32 Public notification.

• • • • •

(b) • • •

(3) Except that the requirements of this subsection (b) may be waived by the State if it determines that the violation has been corrected promptly after discovery, the cause of the violation has been eliminated, and there is no longer a risk to public health.

• • • • •

(d) If a non-community water system fails to comply with an applicable MCL established in Subpart B of this part, fails to comply with an applicable testing procedure established in Subpart C of this part, is granted a variance or an exemption from an applicable MCL, fails to comply with the requirements of any schedule prescribed pursuant to a variance or exemption, or fails to perform any monitoring requirement pursuant to section 1445(a) of the Act, the supplier of water shall give notices by continuous posting of such failure or granting of a variance or exemption to the persons served by the system as long as the failure or granting of a variance or exemption continues. The form and manner for such notices shall be prescribed by the State and shall ensure that the public using the system is adequately informed of the failure or granting of the variance or exemption.

17. Amending Subpart E to read as follows:

Subpart E—Special Monitoring Regulations for Organic Chemicals and Otherwise Unregulated Contaminants

§ 141.41 Special monitoring for sodium.

(a) Suppliers of water for community public water systems shall collect and analyze one sample per plant at the entry point of the distribution system for the determination of sodium concentration levels; samples must be collected and analyzed annually for systems utilizing surface water sources in whole or in part, and at least every three years for systems utilizing solely ground water sources. The minimum number of samples required to be taken by the system shall be based on the number of treatment plants used by the system, except that multiple wells drawing raw water from a single aquifer may, with the State approval, be considered one treatment plant for determining the minimum number of samples. The supplier of water may be required by the State to collect and analyze water samples for sodium more

[3] Techniques of Water—Resources Investigation of the United States Geological Survey, Chapter A-3, "Methods for Analysis of Organic Substances in Water," Book 5, 1972, Stock #2401-1227, Available from Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.

frequently in locations where the sodium content is variable.

(b) The supplier of water shall report to EPA and/or the State the results of the analyses for sodium within the first 10 days of the month following the month in which the sample results were received or within the first 10 days following the end of the required monitoring period as stipulated by the State, whichever of these is first. If more than annual sampling is required the supplier shall report the average sodium concentration within 10 days of the month following the month in which the analytical results of the last sample used for the annual average was received. The supplier of water shall not be required to report the results to EPA where the State has adopted this regulation and results are reported to the State. The supplier shall report the results to EPA where the State has not adopted this regulation.

(c) The supplier of water shall notify appropriate local and State public health officials of the sodium levels by written notice by direct mail within three months. A copy of each notice required to be provided by this paragraph shall be sent to EPA and/or the State within 10 days of its issuance. The supplier of water is not required to notify appropriate local and State public health officials of the sodium levels where the State provides such notices in lieu of the supplier.

(d) Analyses for sodium shall be performed by the flame photometric method in accordance with the procedures described in "Standard Methods for the Examination of Water and Wastewater," 14th Edition, pp. 250–253; or by Method 273.1, Atomic Absorption—Direct Aspiration or Method 273.2, Atomic Absorption—Graphite Furnace, in "Methods for Chemical Analysis of Water and Waste," EMSL, Cincinnati, EPA, 1979; or by Method D1428–64(a) in Annual Book of ASTM Standards, part 31, Water.

18. Adding a § 141.42 to read as follows:

§ 141.42 Special monitoring for corrosivity characteristics.

(a) Suppliers of water for community public water systems shall collect samples from a representative entry point to the water distribution system for the purpose of analysis to determine the corrosivity characteristics of the water.

(1) The supplier shall collect two samples per plant for analysis for each plant using surface water sources wholly or in part or more if required by the State; one during mid-winter and one during mid-summer. The supplier of the water shall collect one sample per plant for analysis for each plant using ground water sources or more if required by the State. The minimum number of samples required to be taken by the system shall be based on the number of treatment plants used by the system, except that multiple wells drawing raw water from a single aquifer may, with the State approval, be considered one treatment plant for determining the minimum number of samples.

(2) Determination of the corrosivity characteristics of the water shall include measurement of pH, calcium hardness, alkalinity, temperature, total dissolved solids (total filterable residue), and calculation of the Langelier Index in accordance with paragraph (c) below. The determination of corrosivity characteristics shall only include one round of sampling (two samples per plant for surface water and one sample per plant for ground water sources). However, States may require more frequent monitoring as appropriate. In addition, States have the discretion to require monitoring for additional parameters which may indicate corrosivity characteristics, such as sulfates and chlorides. In certain cases, the Aggressive Index, as described in paragraph (c), can be used instead of the Langelier Index; the supplier shall request in writing to the State and the State will make this determination.

(b) The supplier of water shall report to EPA and/or the State the results of the analyses for the corrosivity characteristics within the first 10 days of the month following the month in which the sample results were received. If more frequent sampling is required by the State, the supplier can accumulate the data and shall report each value within 10 days of the month following the month in which the analytical results of the last sample was received. The supplier of water shall not be required to report the results to EPA where the State has adopted this regulation and results are reported to the State.

(c) Analyses conducted to determine the corrosivity of the water shall be made in accordance to the following methods:

(1) Langelier Index—"Standard Methods for the Examination of Water and Wastewater," 14th Edition, Method 203, pp. 61–63.

(2) Aggressive Index—"AWWA Standard for Asbestos-Cement Pipe, 4 in. through 24 in. for Water and Other Liquids," AWWA C400–77, Revision of C400–75, AWWA, Denver, Colorado.

(3) Total Filtrable Residue—"Standard Methods for the Examination of Water and Wastewater," 14th Edition, Method 208B, pp. 92–93; or "Methods for Chemical Analysis of Water and Wastes," Method 160.1.

(4) Temperature—"Standard Methods for the Examination of Water and Wastewater," 14th Edition, Method 212, pp. 125–126.

(5) Calcium hardness—EDTA Titrimetric Method "Standard Methods for the Examination of Water and Wastewater," 14th Edition, Method 309B, pp. 202–206; or "Annual Book of ASTM Standards," Method D1126–67 (8).

(6) Alkalinity—Methyl Orange and paint pH 4.5. "Standard Methods for the Examination of Water and Wastewater," 14th Edition, Method 403, pp. 278–281; or "Annual Book of ASTM Standards," Method D1067–70B; or "Methods for Chemical Analysis of Water and Wastes," Method 310.1.

(7) pH—"Standard Methods for the Examination of Water and Wastewater," 14th Edition, Method 424, pp. 460–465; or "Methods for Chemical Analysis of Water and Wastes," Method 150.1; or "Annual Book of ASTM Standards," Method D129378 A or B.

(8) Chloride—Potentiometric Method "Standard Methods for the Examination of Water and Wastewater," 14th Edition, p. 306.

(9) Sulfate—Turbidimetric Method, "Methods for Chemical Analysis of Water and Wastes," pp. 277–278, EPA, Office of Technology Transfer, Washington, D.C. 20460, 1974, or "Standard Methods for the Examination of Water and Wastewater," 13th Edition, pp. 334–335, 14th Edition, pp. 496–498.

(d) Community water supply systems shall identify whether the following construction materials are present in their distribution system and report to the State:

- Lead from piping, solder, caulking, interior lining of distribution mains, alloys and home plumbing.
- Copper from piping and alloys, service lines, and home plumbing.
- Galvanized piping, service lines, and home plumbing.
- Ferrous piping materials such as cast iron and steel.
- Asbestos cement pipe.

In addition, States may require identification and reporting of other materials of construction present in distribution systems that may contribute contaminants to the drinking water, such as:

- Vinyl lined asbestos cement pipe.
- Coal tar lined pipes and tanks.

Appendix A—Response to Public Comments

Comments submitted to the Agency and statements presented at the public hearing in

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Chlorine Determinations and Turbidity

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 75 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: The participant may be responsible for this determination to meet Water Quality Control Program requirements.

V. ENTRY LEVEL BEHAVIOR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will hearn about chlorination products and terminology, and the chemistry involved in chlorine determinations by iodometric analyses and DPD methodology. The participant will know pertinent facts about turbidity and procedures to determine turbidity.

B. Conditions: The participant will be given two outlines: Residual Chlorine and Turbidity (manual) and Chlorine Determinations and Their Interpretation (handout) and 75 minutes of classroom presentation.

C. Accepted Performance: In attendance to the lecture covering subject material.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Amperometric Determination of Total Residual Chlorine (14th edition Standard Methods, page 318.)

2. Calibration and Use of a Turbidimeter (Nephelometer) (1974 EPA Methods for Chemical Analysis, p. 295)

3. Training Manual Outline: Chlorine Determinations and Turbidity

4. Handout Training Outline: Chlorination and Chlorine Determinations

5. Thirty-six slides, X-21: Chlorine (See XI. Description of Visual Materials).

6. Ten slides, X-30: Turbidity (See XI Description of Visual Materials).

B. Suggested Media:

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review available media and visual materials and prepare lesson.

B. Sequencing:

Slides are series X-21: Chlorine

Participants should use the Training Outline, "Chlorination and Chlorine Determinations" as a reference for this information.

1. Classroom instruction on chlorination, using slides to discuss the first 3 Sections of the outline in the training manual.

   a. Introduction about bactericidal use of chlorine - slide 1

   b. Means of chlorination - slide 2

   c. Effects of applying chlorine gas - slide 3, 4

   d. Effects of applying calcium hypochloride - slide 5, 6

   e. Ammonia reactions with hypochlirous acid produce chloramines - slide 7, 8

   f. Side reactions reduce availability of chlorine for disinfecting purposes - slide 9

   g. Chlorination products that have disinfection powers - slide 10

   h. Stress the meaning of the terms related to chlorine residuals - slide 11, 12, 13

   i. Factors affecting disinfection - slide 14

   j. It is convenient to use a blank slide (15) here.

2. Classroom overview of recognized chlorine determinations, using headings in training manual sections on "Iodometric Analyses" and "DPD Methodology", then the summary of "Compliance Methodology".

3. Classroom instruction on the chemistry involved in direct iodometric titrations to determine free, combined or total chlorine. This series of slides presents facts about the chemistry of the method with pictures of an analyst doing each step using an amperometric titrator. Presentation of the direct method gives an opportunity to teach the chemistry involved and also to re-enforce mastery of the meaning of the terms "free", "combined" and "total" residual chlorine. However, for wastewaters the indirect or back titration is to be used. If participants will routinely analyze waste-

water, you can still use these slides to teach the chemistry involved and the meaning of the above terms, but spend more time on the actual steps to do a back titration (next topic).

a. Types of chlorine - slide 16

b. Role of phenylarsin oxide - slide 17

c. Picture of titration equipment - slide 18

d. pH for types - slide 19

e. Principle for free chlorine - slide 20

f. 1 ml pH 7 buffer has been added and current flows, pointer to right - slide 21

g. PAO reduces, current reduced - slide 22

h. Adjust pointer to right - slide 23

i. More PAO, current reduced - slide 24

j. Needle stops, take reading - slide 25

k. More PAO. When needle stays stopped, ti's end. (slide 26 is same as slide 25 to show needle staying same) - slide 26

l. 0.00564N PAO related to chlorine for a 200 ml sample - slide 27

m. PAO related to free chlorine at pH 6.5 - 7.5 = slide 28

n. Combined chlorine may be present - slide 29

o. Utilize chlorine - iodide reaction - slide 30

p. Need pH 4 and potassium iodide - slide 31

q. Combined chlorine releases iodine - slide 32

r. Add KI solution (crystals may be used instead of solution)- slide 33

s. Add pH 4 buffer - slide 34

t. Repeat titration procedure as for free chorine; when needle stays at one place, it is the end point - slide 35

u. Add results for free and for combined chlorine to give total. Stress: can do total in one step by adding KI and pH 4 buffer directly to sample - slide 36

4. Classroom instruction on indirect (back-titration) iodometric procedures to determine total chlorine.

   a. Reason for indirect

   b. Same principles as direct method. Stress additional step using iodine titrant and end point signal is reversed.

5. Classroom instruction on DPD Method

   a. Chemistry involved to produce color

   b. Titration method, stressing end point signal

   c. Spectrophotometric method

6. Summary of methodology recognized for compliance monitoring

Series X30: Turbidity

1. Classroom instruction on Turbidity:

   a. Slide X30-1: Cause

   b. Slide X30-2: Why measure turbidity

   c. Slide X30-3: Holding Time for samples

   d. Slide X30-4: History of Measurement

2. Regulations - NPDES and Water Supply

3. Acceptable instrumentation

   a. Slides X30-5 to X30-6: Nephelometry

   b. Slides X30-7 to X30-9: Formazin Standards

   c. Slide X30-10: Interferences

4. Summary

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom Equipment and Supplies

1. Slide projector and supplies

2. Blackboard and chalk

X. IPW REAGENT REQUIREMENTS:

A. None required

XI. DESCRIPTION OF VISUAL MATERIALS:

A. Series X-21: Chlorine (36 slides)

1. Slide X21-1: Cartoon-type of drawing showing plant operator killing disease organisms with chlorine

2. Slide X21-2: Means of Chlorination - Chlorine gas, Chlorine gas dissolved in water and Calcium hypochlorite

3. Slide X21-3: Effects of Chlorination - Chlorine gas, Chlorine gas dissolved in water→pH Decrease

4. Slide X21-4: Products of Cl_2: H^+ Hydrogen ion, Cl^- Chloride ion, HOCl hypochlorous acid → $H^+ + OCl^-$

5. Slide X21-5: Effects of Chlorination - Calcium hypochlorite → pH Increase

6. Slide X21-6: Products of $Ca(OCl)_2$: Ca^{+2} calcium ion, OH^- hydroxide ion and HOCl hypochlorous acid → $H^+ + OCl^-$

7. Slide X21-7: Ammonia

8. Slide X21-8: Ammonia Reactions with hypochlorite ion - Monochloramines, Dichloramines, and Trichloramines

9. Slide X21-9: Demand from side reactions

10. Slide X21-10: Disinfection - Monochloramines, Dichloramines, and Hypochlorite ion

11. Slide X21-11: Free Chlorine residuals - Chlorine, Hypochlorous acid, and Hypochlorite ion

12. Slide X21-12: Combined Chlorine - Chloramines

13. Slide X21-13: Total residual chlorine = free chlorine residual + combined chlorine residual

14. Slide X21-14: Disinfecting power - Concentration and Contact time

15. Slide X21-15: Blank Slide (dark)

16. Slide X21-16: Free Chlorine - MG/1 + Combined Chlorine - MG/1 ⟶ Total Residual Chlorine

17. Slide X21-17: Reducing Agent/Phenylarsene Oxide

18. Slide X21-18: Live of Titration Assembly

19. Slide X21-19: Two Stage Titration - (1) pH 7 Free Chlorine Residual (2) pH 4 Combined Chlorine Residual

20. Slide X21-20: Current Produced/Proportional to Free Chlorine Present

21. Slide X21-21: Live of Adding Titrant, Pointer to Right

22. Slide X21-22: Live of Adding Titrant, Current Reduced

23. Slide X21-23: Live of Adjusting Pointer to right on scale

24. Slide X21-24: Live of Adding More Titrant

25. Slide X21-25: Live of Pointer at Rest

26. Slide X21-26: Live of Pointer Staying at Rest

27. Slide X21-27: 1 ml Phenylarsene Oxide = 1 mg/l of Chlorine

28. Slide X21-28: Phenylarsene Oxide reacts with Free Chlorine Only

29. Slide X21-29: Free Chlorine/Combined Chlorine

30. Slide X21-30: Starch-Iodide Method
Chlorine Oxidizes Iodide to Produce Free → Iodine

31. Slide X21-31: Combined Chlorine Determination
pH 4 and Addition of Potassium Iodide

32. Slide X21-32: Combined Chlorine Compounds
Oxidize Iodide → Free Iodine

33. Slide X21-33: Live of Adding KI Solution

34. Slide X21-34: Live of Adding pH 4 Buffer

35. Slide X21-35: Live of Repeating Titration Process

36. Slide X21-36: Free Chlorine - mg/l + Combined Chlorine - mg/l
→ Total Residual Chlorine

B. Series X-30: Turbidity (10 slides)

1. Slide X30-1: What is Turbidity - Caused by clay-silt-plankton-other organic and inorganic material and is an optical property - size, shape, specific gravity, number of particles

2. Slide X30-2: Why Measure Turbidity - can prevent proper disinfection practice, persons object to waters with turbidity, can be injurious to industrial processes and equipment and monitor plant operations and filter breakthrough

3. Slide X30-3: Preservation of Turbidity Samples - Must be run as soon as possible - within one hour

4. Slide X30-4: Drawing: Jackson Candle Turbidimeter

5. Slide X30-5: Drawing: Nephelometer Light Path

6. Slide X30-6: Drawing: NephelometerSecondary Light Path

7. Slide X30-7: Formazin Polymer - Use - Solution 1. Hydrazine Sulfate 1.000 gram/100 ml. turbidity free water
Solution 2. Hexamethylene tetramine 10.00 gram/100 ml turbidity free water

8. Slide X30-8: Formazin Polymer - Solution 3 - Add 5.0 ml of solution 1 and 5.0 ml of solution 2 into a 100 ml volumetric flask. Allow to stnad 24 hours at 25 $\pm$ 3°C. Then dilute to 100 ml mark with turbidity free water. Contains 400 NTU suspension.

9. Slide X30-9: Turbidity Free Water - 1. Filter distilled water through membrane filter having pore size $\leq$ 100 µm.
2. Check turbidity of nonfiltered water and filtered water. If filtered water does not have less turbidity use distilled.

10. Slide X30-10: Interferences - Rapid Settling Coarse Sediments, Floating Debris, Air Bubbles, Color, Dirty or Scratched Sample Tubes

Cl Determinations and Their Interpretation - PC.11d.11.77

CHLORINE DETERMINATIONS AND THEIR INTERPRETATION

I INTRODUCTION

Chlorine normally is applied to water as a bactericidal agent; it reacts with water contaminants to form a variety of products containing chlorine. The difference between applied and residual chlorine represents the chlorine demand of the water under conditions specified. Wastewater chlorination is particularly difficult because the concentration of organisms and components susceptible to interaction with chlorine are high and variable. Interferences with the chlorine determination in wastewater confuse interpretation with respect to the chlorine residual at a given time and condition, its bactericidal potency, or its future behavior.

II CHEMISTRY OF CHLORINATION

A Chlorine compounds (Cl_2) dissolve in water, and hydrolyze immediately according to the reaction.

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^-$$

The products of this reaction are hypochlorous and hydrochloric acid. The reaction is reversible, but at pH values above 3.0 and concentrations of chlorine below 1000 mg/l the shift is predominantly to the right leading to hypochlorous acid (HOCl).

Hypochlorous acid is a weak acid and consequently ionizes in water according to the equation:

$$HOCl \rightleftharpoons H^+ + OCl^-$$

This reaction is reversible. At a pH value of 5.0 or below almost all of the chlorine is present as hypochlorous acid (HOCl) whereas above pH 10.0 nearly all of it exists as hypochlorite ion (OCl^-). The pH value that will control is the pH value reached after the addition of chlorine. Chlorine addition tends to lower the pH and the addition of alkali hypochlorites tends to raise the pH.

B The initial reactions on adding chlorine to wastewaters may be assumed to be fundamentally the same as when chlorine is added to water except for the additional complications due to contaminants and their concentration.

Hypochlorous acid (HOCl) reacts with ammonia and with many other complex derivatives of ammonia to produce compounds known as chloramines. Formation of the simple ammonia chloramines includes:

1 $NH_3 + HOCl \rightarrow NH_2Cl + H_2O$

monochloramine

2 $NH_2Cl + HOCl \rightarrow NHCl_2 + H_2O$

dichloramine

3 $NH_2Cl + NHCl_2 \rightarrow N_2 + 3\ HCl$

The distribution of the ammonia chloramines is dependent on pH, as illustrated below:

ph	Percentage of Chlorine Present as Monochloramine	Dichloramine
5	16	84
6	38	62
7	65	35
8	85	15
9	94	6

The formation of the ammonia chloramines are dependent on pH, temperature, and chlorine-ammonia ratio. Chlorine reactions with amino acids are likely; product disinfecting powers are lower than those of chlorine or of ammonia chloramines.

III TERMINOLOGY

A Terms used with Respect to Application Site

1 Pre-chlorination - chlorine added prior to any other treatment.

2 Post-chlorination - chlorine added after other treatment.

3 Split chlorination - chlorine added at different points in the plant - may include pre- and post-chlorination.

B Terms Used in Designating Chlorine Fractions

1 Free available residual chlorine - the residual chlorine present as hypochlorous acid and hypochlorite ion.

2 Combined available residual chlorine - the residual chlorine present as chloramines and organic chlorine containing compounds.

3 Total available residual chlorine - the free available residual chlorine + the combined available residual chlorine - may represent total amount of chlorine residual present without regard to type.

In ordinary usage these terms are shortened to free residual chlorine, combined residual chlorine and total residual chlorine. In the chlorination of wastewaters only combined residual chlorine is ordinarily present and is often improperly termed chlorine residual.

C Breakpoint chlorination specifically refers to the ammonia-chlorine reaction where applied chlorine hydrolyzes and reacts to form chloramines and HCl with the chloramines eventually forming N_2 + HCl as in II. B. 3. Assuming no other chlorine demand, the total chlorine residual will rise, decrease to zero and rise again with increasing increments of applied chlorine. Other substances may produce humps in the applied chlorine vs residual chlorine plot due to oxidation of materials other than ammonia. Sometimes these are erroneously considered as a breakpoint.

IV IODOMETRIC TITRATION ANALYSES[1, 2]

Iodometric titration using either an amperometric or a starch-iodine end point determines chlorine residual. The relative advantages of a specific determination depend upon the form in which the reactable chlorine exists and the amount and nature of interferences in the water.

A Amperometric Titration - Direct Method

1 Scope and application

This method is applicable to the determination of free, combined or total residual chlorine in all types of water and wastewaters that do not contain substantial amounts of organic matter.

2 Summary of Method

When the cell of the titrator is immersed in a sample at pH 7.0, the cell unit produces a small direct current if free chlorine (an oxidizing agent) is present. As phenylarsine oxide (PAO) solution is added, it reduces the free chlorine. When all the chlorine is neutralized, the generation of current ceases. At this end point, the microammeter pointer on the apparatus no longer deflects down-scale.

To determine combined chlorine, pH 4.0 buffer and potassium iodide are then added to the sample. Free iodine released by the combined chlorine also causes the cell to produce a small direct current. Addition of PAO reduces the free iodine and the generation of current ceases. Again, the end point occurs

when the microammeter pointer no longer deflects down-scale.

In either titration, the amount of PAO reducing agent used to reach the end point is ultimately stoichiometrically proportional to chlorine present in the sample. The sum of the free and combined chlorine is the total residual chlorine in the sample.

Total residual chlorine can be determined directly by adding pH 4.0 buffer and potassium iodide to the sample before beginning the titration. Any free or combined chlorine present will stoichiometrically liberate free iodine which is then reduced with PAO titrant. The amount of PAO titrant used measures the total amount of free and combined chlorine originally present in the sample.

3 Interferences

a Organic matter reacts with liberated iodine.

b Cupric ions may cause erratic behavior of the apparatus.

c Cuprous and silver ions tend to poison the electrode by plating out on it.

B Amperometric Titration - Indirect Method

1 Scope and Application

This method is applicable to the determination of total chlorine residual in all types of water and wastewaters. In contrast to the direct amperometric titration, this back-titration procedure minimizes interferences in waters containing substantial amounts of organic matter.

2 Summary of Method

A sample is treated with a measured excess of standard phenylarsine oxide (PAO) solution followed by addition of potassium iodide and a buffer to maintain the pH between 3.5 and 4.2. Any form of chlorine present will stoichiometrically liberate iodine which immediately reacts with the PAO before significant amounts are lost to reactions with organic matter in the sample.

When the cell of the amperometric titrator is immersed in a sample so treated, no current is generated since neither free chlorine nor free iodine is present.

The amount of PAO used to reduce the liberated iodine is then determined by titrating the excess with a standard iodine solution. No current is generated until all the excess PAO has been oxidized by the iodine. At this end point the next small addition of iodine causes current to be generated and the microammeter pointer permanently deflects up-scale.

The excess PAO thus measured is subtracted from the original amount of PAO added. The difference is the PAO used to reduce the liberated iodine and is ultimately a measure of the total chlorine originally present in the sample.

NOTE: Sodium thiosulfate solution may be used instead of PAO, but PAO is more stable and is to be preferred.

3 Interferences

a Cupric ions may cause erratic behavior of the apparatus.

b Cuprous and silver ions tend to poison the electrode by plating out on it.

C Colorimetric Starch-Iodide Titration - Indirect Method

1 Scope and Application

This method is applicable to the determination of total chlorine residual in all types of water and wastewater. A back-titration procedure is used to minimize interferences in waters containing substantial amounts of organic matter.

2 Summary of Method

A sample is treated with a measured excess of standard phenylarsine oxide (PAO) solution followed by addition of potassium iodide and a buffer to maintain the pH between 3.5 and 4.2. Any form of chlorine present will stoichiometrically liberate iodine which immediately reacts with PAO before significant amounts are lost to reactions with organic matter in the sample.

The amount of PAO used to reduce the liberated iodine is then determined by titrating the excess with a standard iodine solution in the presence of starch until the PAO is completely oxidized. At this end point, the next small addition of iodine causes a faint blue color to persist in the sample.

The excess PAO thus measured is subtracted from the original amount of PAO added. The difference is the PAO used to reduce the liberated iodine and is ultimately a measure of the total chlorine originally present in the sample.

NOTE: Sodium thiosulfate solution may be used instead of PAO, but PAO is more stable and is to be preferred.

3 Interferences

a An unusually high content of organic matter may cause some uncertainty in the end point. If manganese, iron and nitrite are definitely absent, this uncertainty can be reduced by acidification to pH 1.0.

b Color and turbidity in the sample cause difficulty with end-point detection.

D Evaluation of Iodometric Analyses

Iodometric titration using amperometric end point detection appears to be the more accurate residual chlorine method. Besides being inherently more accurate than color detection methods, this electrical end point is free of interference from color and turbidity.

The accuracy of the colorimetric starch-iodide end point is improved by employing the indirect titration method described above. By adding an excess of the standard reducing agent and back-titrating, contact between the liberated iodine and organic matter in the sample is minimized.

V DPD METHODOLOGY[1]

The DPD (N, N-diethyl-p-phenylenediamine) method can be applied by either titration or spectrophotometry.

A Titration

In this procedure, the buffered sample is titrated with ferrous ammonium sulfate to the disappearance of the red color; the result is free available chlorine. Using the same sample, the titration can be carried further to determine mono-and dichloramine. Nitrogen trichloride is found using a fresh portion of sample.

B Spectrophotometry

Alternatively, the red colors in A can be read in a spectrophotometer or filter photometer at 515 nm. The concentrations are determined using a calibration graph.

VI COMPLIANCE METHODOLOGY

A NPDES/Certifications

All of the analytical procedures described in IV, Iodometric Titration Analyses and in V, DPD Methodology (above) are cited in the Federal Register as approved.

B Drinking Water

Only the DPD Methodology (as in V above) is cited in the Federal Register as approved. In contrast to the NPDES/ Certifications regulations, the DPD test kit is also approved for drinking water.

ACKNOWLEDGEMENT:

Significant portions of this outline were written by J. L. Holdaway, Chemist, Technical Program, USEPA, Region III, Charlottesville, VA 22901 (1971) and by Charles R. Feldmann, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268.

REFERENCES

1 Standard Methods for the Examination of Water and Wastewaters, 14th Ed., APHA, AWWA, WPCF. 1976.

2 Book of ASTM Standards, Part 31, Water, American Society for Testing and Materials, Philadelphia, PA. 1975.

3 Sawyer, C. N. Chemistry for Sanitary Engineers. McGraw-Hill Book Company. New York. 1960.

4 Moore, E. W. Fundamentals of Chlorination of Sewage and Wastes. Water and Sewage Works. Vol. 98. No. 3. March 1951.

5 Day, R. V., Horchler, D. H., and Marks, H. C. Residual Chlorine Methods and Disinfection of Sewage. Industrial and Engineering Chemistry. May 1953.

6 Marks, H. C., Joiner, R. R., and Strandskov, F. B. Amperometric Titration of Residual Chlorine in Sewage. Water and Sewage Works. May 1948.

This outline was updated by Audrey D. Kroner, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Chemical Analysis, Chlorination, Chlorine, Disinfection, Sewage Treatment, Wastewater Treatment, Water Analysis.

GUIDELINES FOR

INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Bacteriological Indicators of Water Quality

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 90 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: Coverage of this subject matter gives the participant an insight to the development of the rationale concerning the concept of an ideal indicator and the indicator bacteria in use today for various objectives of Water Quality Programs.

V. ENTRY LEVEL BEHAVIUR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be aware of the current bacteriological indicators used in Water Quality Programs and the advantages/limitations inherant to their use.

B. Conditions: Instructional material covered in course training manual.

C. Accepted Performance: In attendance to the lecture material covering subject material.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training Manual Outline: "Bacteriological Indicators of Water Pollution".

2. 53 Slides of X39 Series.

B. Suggested Media:

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review visual and written material and prepare lesson.

2. Sequence slides as desired and place in slide tray.

3. Prepare any handout material, if desired, and arrange to have an adequate supply prepared.

4. Assemble any desired demonstration equipment/materials and arrange to have them available for the presentation.

B. Sequencing:

1. Classroom presentation of X39

a. Two areas of approach to finding a possible bacteriological indicator of water quality. (X39-1)

b. Normal and pathogenic flora in human feces. (X39-2)

c. Use of "total count" as an indication of water quality. (X39-3)

d. Early Bacterial Criteria Relating to Sanitary Water Quality . . . indication that investigators widely disagreed as to water quality vs. total counts. (X39-4)

e. Concept of an "Ideal Indicator". 8 Slides (X39-5 to X39-12).

f. Concept of using pathogens is indicators of water quality. 6 Slides (X39-13 to X39-18).

g. Escherichs' discovery of Gram Negative rods in genral population and subsequent advancement of the coliform group of bacteria as indicators of Water Quality. (X39-19)

h. Current coliform group definition. (X39-20)

i. Limitations of coliform group. 2 Slides (X39-21 to X39-22).

j. IMV;C Classification. 6 Slides (X39-23 to X39-28).

k. Coliforms as Indicators Advantages/Limitations. (X39-29)

l. Escherichеae - Genus and Habitats (X39-30)

m. Effects of temperature on the Colifrom bacteria. 3 Slides (X39-31 to X39-33).

n. Fecal Coliform Significance (X39-34)

o. Fecal Coliform NTAC Findings (X39-35)

p. Fecal Coliform Standard Test Procedures. (X39-36)

q. Fecal Coliform Indicators Advantages/Limitations (X39-37)

r. Groups of Enteric Bacteria Total Coliform and Fecal Coliform zones. (X39-38)

s. Fecal Streptococci - Definition. 3 Slides (X39-39 to X39-41).

t. Fecal Streptococci - Slow acceptance as indictors. (X39-42)

u. Use of Fecal Streptococci as indicators. 3 Slides (X39-43 to X39-45).

v. Partition Counting Principles. 4 Slides (X39-46 to X39-49).

w. Fecal Coliform to Fecal Streptococci Ratio. 4 Slides (X39-50 to X39-53).

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom Equipment and Supplies:

1. Slide projector and tray.

2. Blackboard and chalk.

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS:

A. 53 Slides, X39: Bacteriological Indicators of Water Quality

1. Slide X39-1 Bacterial Indicators of Pollution - Two Areas of Possibility

2. Slide X39-2 Normal and Pathogenic Flora in Human Feces

3. Slide X39-3 Total Count Plate

4. Slide X39-4 Early Bacterial Criteria Relating To Sanitary Water Quality

5. Slide X39-5 Ideal Indicators: Lack Laboratory Safety Hazard

6. Slide X39-6 Ideal Indicator: Positive Test = Pollution

7. Slide X39-7 Ideal Indicator: Negative Test = No Pollution

8. Slide X39-8 Ideal Indicator: Uniform Results with Different Water

9. Slide X39-9 Ideal Indicator: Never Present in Safe Water

10. Slide X39-10 Ideal Indicators Show No Aftergrowth in Stream

11. Slide X39-11 Ideal Death Curves Indicator - Pathogenic Microorganisms

12. Slide X39-12 Characteristics of an Ideal Indicator

13. Slides X39-13 Bacteriologic Diagnosis of Specimens for Bacillary Dysentery Shigellosis).

14. Slide X39-14 Bacteriologic Diagnosis of Specimens for Brucellosis

15. Slide X39-15 Microscopic appearance of protozoan forms

16. Slide X39-16: Bacteriological Cultures Undergoing Biochemical Examination.

17. Slide X39-17: Pathogens as Indicators Advantages/Limitations.

18. Slide X39-18: Bacteriological Measurements of Fecal Pollution.

19. Slide X39-19: Escherich's Examinations of Ward Patients Discovers Ubiguitous Gram-Negative rods in fecal specimens

20. Slide X39-20: Coliform Group Definition.

21. Slide X39-21: Relationship Between Coliform Concentrations and Rainfall.

22. Slide X39-22: Effect of Rainfall and Surface Runoff on Coliform Bacteria in the Missouri River Below Kansas City.

23. Slide X39-23: Differentiation Tests for Coliforms.

24. Slide X39-24: Known IMViC Types.

25. Slide X39-25: A. Aerogenes Type 1 IMViC and EC Reactions.

26. Slide X39-26: E. Coli Type 1 IMViC and EC Reactions.

27. Slide X39-27: Coliforms 67 Soil Samples IMViC Reactions.

28. Slide X39-28: IMViC Reactions of Warm Blooded Animal Cultures.

29. Slide X39-29: Coliforms as Indicators Advantages/Limitations.

30. Slide X39-30: Habitats of Escherichear

31. Slide X39-31: Citrate Positive vs Citrate Negative Cultures and Their Lactose Reactions at Varying Temperatures.

32. Slide X39-32: Averaged Generation Times, 0 to 6 hours, for Coliforms in seven hour liquid median.

33. Slide X39-33: Fecal Coliform and Non-fecal Coliform Growith Response in EC both at various temperatures.

34. Slide X39-34: Fecal Coliform Significance.

35. Slide X39-35: Fecal Coliforms-NTAC Findings.

36. Slide X39-36: MPN and MF Techniques at the Elevated Temperature for Fecal Coliforms.

37. Slide X39-37: Fecal Coliform Indicators Advantages/Limitations.

38. Slide X39-38: Groups of Enteric Bacteria-Placement of Coliform and Fecal Coliforms.

39. Slide X39-39: Fecal Streptococci Definition.

40. Slide X39-40: Fecal Streptococci of Sanitary Significance.

41. Slide X39-41: Classification of Streptococci.

42. Slide X39-42: Fecal Streptococci Slow Acceptance as Indicators.

43. Slide X39-43: Fecal Streptococci Sanitary Interpretations.

44. Slide X39-44: Occurence of S. fecalis var liquefaciens in Various Environmental Sources.

45. Slide X39-45: S. bovis - S. equinus Percentage of Fecal Streptococcus Population in Warm Blooded Animal Feces.

46. Slide X39-46: Occurrence of Streptococcal Groups in Feces of Man, Cow, Sheep, Pig and Fowl.

47. Slide X39-47: Distribution of Fecal Streptococcal Groups in Feces of Humans.

48. Slide X39-48: Distribution of Fecal Streptococcal Groups in Feces of Cows.

49. Slide X39-49: Distribution of Fecal Streptococci in Sewage and in Human and Cow Feces.

50. Slide 39-50: Indicator Densities per Capita per day in decending order.

51. Slide X39-51: Estimated per Capita Contribution of Indicators of Some Common Animals and its FC/FS Ratio.

52. Slide X39-52: Bacteriological Quality of Irrigation Water.

53. Slide X39-53: Applying the FC/FS Ratio.

SUBJECT MATTER: Advance preparations for Day One Laboratory

WHEN ACCOMPLISHED: Approximately 24 hours prior to Day One Laboratory and 2 hours prior to Day One Laboratory

ACCOMPLISHED BY: Qualified instructor

TIME REQUIRED: 3-5 hours (dependent on the time required to sample and the number of students enrolled)

PREPARATION REQUIRED	REMARKS
24 hours advance	
Inoculate test tube rack containing lactose broth and AD broth tubes. One rack per student is required.	Each rack to contain 25 lactose and 25 AD tubes (first row multiple strength and the other 4 rows single strength). Each rack constitutes an MPN test to be assigned to each student. When inoculated, incubate at 35C ± 0.5C.
Prepare and plate MFC medium with sample volume which will produce isolated colonies.	At least one plate for each 2 students. Incubate at 44.5C ± 0.2C. To be used for Verification Test to be initiated by students in Day One Laboratory.
2 hours advance	
Have laboratory operational for laboratory studies.	1. Test Rack of sterile lactose tubes/trainee 2. Appropriate data sheets/trainee 3. MPN transfer equipment/trainee 4. Sterile BGB, EC, lactose, and EVA Supply 5. MF equipment (media preparation; preweighed LES & m-ENDO media; MF plates) 6. Verification in test equipment 7. 35°C and 44.5°C incubators operational 8. Place one rack/trainee of the 24 hour MPN tubes at each laboratory position 9. Remove MFC plates from 44.5°C incubator and have available for verification test procedure

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Laboratory Briefing for Day One

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 40 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: Briefing for this variety of subject matter, as described under VII Available Media, is of primary importance since a number of procedures will be initiated without benefit of prepatory lecture groundwork due to time constraints.

V. ENTRY LEVEL BEHAVIOR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be acquainted with the various areas of instructional material covered in the training course manual and better be able to utilize them in an open book laboratory situation. which immediately follows the briefing session.

B. Conditions: Instructional material as covered in various parts of course training manual and as directed by verbal instructions.

C. Accepted Performance: Utilization of assigned pages of course training manual in conjunction with notes taken during briefing sessions.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training Manual Outlines:

Outline	Section	Purpose
MPN Methods	Tube Labeling	Instructor Aid-Tubes labeled to help preclude tube manipulation errors by trainee during course.
MPN Methods	Tube Sample Inoculations (pipeting)	Provides accurates distribution of correct sample volumes to each tube.
MPN Methods	Preparation of dilutions	Provides accurate measurement of required sample dilutions.
MPN Methods	Culture Transfers	Provides correct technique of culture transfer and approved methods.

Outline	Section	Purpose
Detailed MF Methods	M·ENDO medium preparation	Provides correct technique of medium preparation
Verified MF Tests	Verification of fecal coliforms	Provides technique and schematic for test procedure

2. Suggested Media:

   A. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review agenda for time sequencing and written material for content and prepare briefing.

2. Prepare handout (or use blackboard, if desired) indicating to trainee the written material to review. An example of such a handout would be as follows:

Item	Outline and Pages	Standard Methods(14th Edition)
Tube Labeling	MPN Methods Pt. 2 review entire section (3-11 to 3-21)	Not specifically covered
MPN Sample Inoculations (Pipeting)	MPN Methods Pt. 1 3-4 to 3-6; 3-11 to 3-12; 3-23	882-883; 916; 943
MPN Dilutions	MPN Methods Pt. 1 3-6, 3-7	883; 892; 909; 910
MPN Culture Transfers	MPN Methods Pt. 1 3-6; 3-7. Pt. 2 3-14; 3-15; 3-17; 3-18; 3-20; 3-23; 3-24	883; 884; 917; 922; 943
M-ENDO Preparation	Detailed MF Methods 11-1; 11-5; 11-7	895
Verification of Fecal Coliforms	Verified MF Tests 13-2; 13-3	No specific information

B. Sequencing:

1. Verification Test

2. m-ENDO preparation

3. MPN information

4. Instructor will then demonstrate the 24 hr procedures with a hypothetical rack of tubes. This will clarify, if needed, the information given in (3) and will serve as a test that the instructor will carry through during the week.

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom Equipment and Supplies:

1. Blackboard and chalk.

2. Demonstration setup.

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS

A. None required.

TRAINEE

MPN TEST PROCEDURE

1. Inoculate sample rack containing lactose tubes with a test sample

   Intended for practice only to learn pipetting procedures.

2. Read and Record a 24 hour fermentation tube rack. Use the form provided by the facility. Record for both the LST tubes and the AD tubes.

   Tubes were inoculated by instructor during advance preparations.

3. Transfer positive tubes to BGB and EC for the Total coliform and Fecal coliforms respectively

   Utilize transfer options for the positive tubes as instructed.

4. Transfer positive tubes of AD to the EVA medium · Do not discard postive AD tubes.

5. Incubate at 35C when all transfers are complete. The EC medium is incubated at 44.5C. Mark tubes to avoid loss or misreading.

6. Prepare 100ml of m-ENDO broth and 30ml of LES ENDO Agar. From these simultaneously prepared media, prepare 6 plates from each to be labeled by the maker and stored for later use in the refrigerator. Save the remainder of the 100ml m-ENDO broth preparation for later use (label this bottle with your initials)

MF Procedure: Pick 6 colonies from MFC plates. Pick typical FC colonies and some non-coliforms. Use sterile needle and LST tubes.

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: The Membrane Filter

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 45 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: Knowledge of this material gives the participant a historical and practical knowledge of the membrane filter allowing its optimal utilization in laboratory operations.

V. ENTRY LEVEL BEHAVIOR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will have knowledge of the advantages and limitations of the membrane filter and its proper handling during sterilization and laboratory manipulations.

B. Conditions: Instructional material as covered in course training manual.

C. Accepted Performance: In attendence to the lecture covering subject material.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training Manual Outline: The Membrane Filter in Water Bacteriology.

2. 12 Slides of X41 Series.

B. Suggested Media:

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review visual and written material and prepare lesson.

2. Sequence slides as desired and place in slide tray.

3. Assemble any desired demonstration equipment/materials and arrange to have them available for the presentation.

B. Sequencing:

1. Classroom presentation of X41.

a. Fiat Final Report on the feasibility of using membrane filters for bacteriological operations. (X41-1)

b. Comparison of similar magnification of membrane filter and a high quality filter paper (X41-2 to X41-3).

c. Drawing of damage inflicted on a membrane filter surface by a camel hair brush. (X41-4)

d. Various materials used for the manufacture of membrane filters. (X41-5)

e. Quality Control Tests used on membrane filters. (X41-6)

f. Flow Rate - definition and a typical chart of values. (X41-7 to X41-8).

g. Membrane Filter - Open volume to solid material and pore size variation. (X41-9)

h. Membrane filter made transparent with use of suitable oils. (X41-10)

i. Differential absorption of dyes by the membrane filter. (X41-11)

j. Membrane Filter sterilization procedures. (X41-12)

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom Equipment and Supplies:

1. Slide projector and tray.

2. Blackboard and chalk.

X. IPW REAGENT REQUIREMENTS:

A. None

XI. DESCRIPTION OF VISUAL MATERIALS

A. 12 Slides, X41: The Membrane Filter

1. Slide X41-1 Fiat Final Report Summary of Document 1312 (8Dec 47)

2. Slide X41-2 Photomicrograph of High Quality Filter Paper

3. Slide X41-3 Photomicrograph of Membrane Filter

4. Slide X41-4 Drawing of Membrane Filter Surface Damage by Passage of a Camel Hair Brush

5. Slide X41-5 Membrane Filter Materials

6. Slide X41-6 Typical Membrane Filter Quality Control Tests by Manufacturer

7. Slide X41-7 Definition of Flow Rate

8. Slide X41-8 Typical Flow Rate Chart

9. Slide X41-9 Schematic: Membrane Filter Open Volume to Solid Material
   Curve: Membrane Filter Pore Size Distribution Curve for
   .45 micron pore diameter

10. Slide X41-10 Membrane Filter clarification with appropriate oil

11. Slide X41-11 Selective absorption of dyes by the membrane filter

12. Slide X41-12 Membrane Filter Sterilization

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Membrane Filter Equipment and Its Preparation for Laboratory Use

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 45 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: Knowledge of this material will allow the participant to be aware of required membrane filtration equipment and its preparation for laboratory usage.

V. ENTRY LEVEL BEHAVIOR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will have knowledge of the types, handling, and preparation of membrane filter laboratory equipment.

B. Conditions: Instructional material as covered in course training manual and the availability of the current edition of Standard Methods for the Examination of Water and Wastewater for reference.

C. Accepted Performance: In attendance to the lecture covering subject material. Course examination may contain questions regarding this subject.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training Manual Outline: "Membrane Filter Equipment and Its Preparation for Laboratory Use"

2. Standard Methods for the Examination of Water and Wastewater (for the 14th Edition: Pgs. 880-886; 891-892, 928-931)

B. Suggested Media:

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review Training Manual Outline and pertinent pages of Standard Methods and prepare lesson.

B. Sequencing:

1. A convenient sequencing for presentation of this subject material is that which follows from the training manual outline. This will also allow the participants to raise questions with the sequential progression through the pages of the outline which should have been read by the participants.

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom Equipment and Supplies

1. Blackboard and Chalk

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS:

A. None required.

Instructor may elect to show materials and equipment during the presentation. Possible examples of these are:

1. Manufactures maintenance kits
2. Various funnel units
3. Wrappings used for sterilization
4. Vacuum sources (asperators, pumps, etc.)
5. Suitable and prohibited types of culture containers

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Examination of Water for Coliform and Fecal Streptococcal Groups (MPN)

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 90 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: Knowledge of this material allows the participant to have a theoretical knowledge and an overview of the MPN test procedure which is a basic test procedure for bacteriological examination of water and wastewater.

V. ENTRY LEVEL BEHAVIOR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be knowledgable in the theoretical aspects of this test procedure and have an overview of the complete procedure.

B. Conditions: Instructional material as covered in course training manual.

C. Accepted Performance: In attendance to the lecture material covering subject material.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training manual outline: "Examination of Water for Coliform and Fecal Streptococcal Groups"

2. 40 slides of X40 Series

3. Standard Methods (for the 14th Editon: pgs: 913-927; 942-944).

B. Suggested Media:

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review all written and visual material and prepare lesson.

2. Sequence slides as desired and place in slide tray.

3. Prepare any handout material, if desired, and arrange to have an adequate supply prepared.

4. Arrange to have any desired demonstration material available for the presentation.

B. Sequencing:

1. Classroom presentation of X40:

a. Schematic overview of MPN Test for Coliform Group. Slide X40-1.

b. Coliform Group definition. Slide X40-2.

c. Factors effecting results of MPN test. Slides X40-3 to X40-6.

d. Sample inoculations. Slides X40-7 to X40-10.

e. Sample volume vs. Range Covered. Slide X40-11.

f. Schematic: Presumptive Test. Slide X40-12.

g. Pictorial Representation of Presumptive test. Slide X40-13.

h. Incubation of tubes at 35° C. Slide X40-14.

i. Inspection and typical recording of 24 hour Presumptive Test tubes. Slides X40-15 to X40-16.

j. Schematic: Confirmed Test. Slide X40-17

k. Loop transfer of culture. Slides X40-18 to X40-19.

l. Continuation of Confirmed Test Procedure. Slides X40-20 to X40-21.

m. Selecting Codes for MPN calculations. Slide X40-22.

n. MPN Table. Slide X40-23.

o. Completed Test Procedure Overview. Slides X40-24 to X40-25.

p. EMB Agar-plating and colonies. Slides X40-26 to X40-29.

q. Completed Test Procedure. Slides X40-30 to X40-34.

r. Fecal Streptococci MPN Schematic. Slide X40-35.

s. Control and Positives for the Fecal Streptococci MPN. Slide X40-36.

t. Fecal Coliform MPN Test. Schematic and Description of Analysis. Slides X40-37 to X40-38.

u. Fecal Coliform Test. First Stage and Second Stage. Slides X40-39 to X40-40.

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom Equipment and Supplies:

1. Slide Projector and supplies.

2. Blackboard and chalk.

3. Demonstration cultures, if desired.

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS

A. 40 Slides, X40: Examination of Water for Coliform and Fecal Streptococcal Groups (MPN)

1. Slide X40-1: Schematic-Tests for Coliform Group.

2. Slide X40-2: Coliform Group Definition.

3. Slide X40-3: Shaking of Sample Bottle to Obtain Even Distribution of Bacteria.

4. Slide X40-4: Poor Selection of Sample Volumes-Lack of Positives in Confirmed Test.

5. Slide X40-5: Poor Selection of Sample Volumes-All positive in Confirmed Test.

6. Slide X40-6: MPN Test. 95% Confidence Limits decrease with selection of at least 5 tables/row.

7. Slide X40-7: MPN Sample Inoculation-Use of Pipet.

8. Slide X40-8: MPN Sample Inoculation-Schematic for dilutions.

9. Slide X40-9: MPN Sample Inoculation-Dilution Blanks (99 ml).

10. Slide X40-10: MPN Sample Inoculation-Use of Pipet.

11. Slide X40-11: Sample Volumes vs. Range Covered.

12. Slide X40-12: MPN Schematic-The Presumptive Test Procedure.

13. Slide X40-13: MPN Pictorial Representation-Presumptive Test Procedure.

14. Slide X40-14: Incubation at 35° C.

15. Slide X40-15: MPN Presumptive Test-24 hour cultures.

16. Slide X40-16: Example of a 24 hour recording for the Presumptive Test.

17. Slide X40-17: MPN Schematic-The Confirmed Test Procedure.

18. Slide X40-18: Cross-Section of a Filled Loop.

19. Slide X40-19: MPN Confirmed Test Loop Transfers.

20. Slide X40-20: MPN Confirmed Test-Inspection of 48 hour test results.

21. Slide X40-21: MPN Confirmed Test-Reincubation of 24 hour negative BGB tubes.

22. Slide X40-22: Selecting MPN Codes from Data Sheet.

23. Slide X40-23: MPN Table (13th Edition of Standard Methods).

24. Slide X40-24: MPN Schematic-The Completed Test Procedure.

25. Slide X40-25: Pictorial Representation of MPN Completed Test Procedure.

26. Slide X40-26: Streaking and EMB Agar Plate.

27. Slide X40-27: Colonies on EMB Agar Plate.

28. Slide X40-28: Same as above.

29. Slide X40-29: Same as above.

30. Slide X40-30: Picking pure cultures from an EMB Agar plate.

31. Slide X40-31: Transferring a pure culture during the MPN Completed Test Procedure.

32. Slide X40-32: Growth of pure culture during the MPN Completed Test Procedure.

33. Slide X40-33: Gram Staining Reagents.

34. Slide X40-34: Gram Positive Culture.

35. Slide X40-35: Schematic: MPN Fecal Streptococcus Test.

36. Slide X40-36: Control and Positive Cultures in the MPN Fecal Streptococcus Test Procedure.

37. Slide X40-37: Schematic-the MPN Fecal Coliform Test.

38. Slide X40-38: Description of the MPN Fecal Coliform Test Procedure.

39. Slide X40-39: MPN Fecal Coliform Test First Stage.

40. Slide X40-40: MPN Fecal Coliform Test Second Stage.

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Media and Solutions for MPN Methods

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 50 minutes

IV. JUSTIFICATION FOR THIS INSTRUCITONAL OBJECTIVE: Knowledge of this material will allow the participant to be aware of required MPN media and solutions and its preparation for laboratory use.

V. ENTRY LEVEL BEHAVIOR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will have knowledge of the required MPN media and solutions and their preparations.

B. Conditions: Instructional material as covered in course training manual and the availability of the current edition of Standard Methods for the Examination of Water and Wastewater for reference.

C. Accepted Performance: In attendance to the lecture covering subject material. Course examination may contain questions regarding this subject.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training manual outline: "Media and Solutions for Multiple Dilution Tube Methods".

2. Standard Methods for the Examination of Water and Wastewater (for the 14th Edition: Pgs. 886-891; 892-896; 918-919).

B. Suggested Media:

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review training manual outline and pertinent pages of Standard Methods and prepare lesson.

B. Sequencing:

1. A convenient sequencing for presentation of this subject material is that which follows from the training manual outline. This will

also allow the participants to raise questions with the sequential progression through the pages of the outline which should have been read by the participants.

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. For each student:

1. Classroom equipment and supplies

2. Blackboard and chalk

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS:

A. None required.

Instructor may elect to show materials and supplies during the presentation. Possible examples of these are:

1. Jars of dehydrated media

2. Bottles of dyes and reagents

3. Stock and working solutions

4. Prepared media

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Principles of Culture Media For Use With Membrane Filters

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 45 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: Knowledge of this material will allow the participant to be aware of principles of culture media and better exercise judgement to protect the integrity of the laboratory preparations.

V. ENTRY LEVEL BEHAVIOR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be aware of the principles of culture media and of some of the terminology applied to various test procedures using differing media preparations.

B. Conditions: Instructional material as covered in course training manual and the availability of the current edition of Standard Methods for the Examination of Water and Wastewater for reference.

C. Accepted Performance: In attendance to the lecture covering subject material. Course examination may contain questions regarding this subject.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training Manual Outline: "Principles of Culture Media for Use With Membrane Filters"

2. Standard Methods for the Examination of Water and Wastewater (for the 14th Edition: Pgs. 882-884; 886-888; 894-895)

B. Suggested Media:

None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review Training Manual Outline and pertinent pages of Standard Methods and prepare lesson.

B. Sequencing:

A convenient sequencing for presentation of this subject material is that which follows from the training manual outline. This will also allow the participants to raise questions with the sequential progression through the pages of the outline which should have been read by the participants.

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom Equipment and Supplies

1. Blackboard and Chalk

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS:

A. None required.

SUBJECT MATTER: Advance Preparations for Day Two Laboratory

WHEN ACCOMPLISHED: Approximately 1 hour prior to Day Two Laboratory

ACCOMPLISHLD BY: Staff

TIME REQUIRED: 1-2 hours

PREPARATION REQUIRED	REMARKS
Remove 48 hour MPN Tests from incubators and place each test rack at the proper test position (bench site)	Individual racks should have been labeled as to trainee identification. If tubes were consolidated due to incubator space, have racks available for participants to obtain theirs.
Have sufficient sterile BGB, EC, and EVA tubes available in laboratory	Required for 48 hour MPN procedures. EC also for verification test
Collect sample for MF Testing	Several liters collected, well mixed, and placed in individual single bottles for trainees to run MF test
Place m-ENDO broth at each laboratory position	Was prepared individually on Monday (Day One) and stored in refrigerator and should have been labeled as to trainee identification
Have laboratory operational for laboratory studies	1. MPN Transfer equipment/trainee 2. Sterile BGB, EC, and EVA tubes available 3. MF test equipment/supplies at each laboratory position 4. MF Data Sheets distributed 5. All incubators operational

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Laboratory Briefing for Day Two

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 45 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: To ensure understanding of previous laboratory work and to give a briefing for the work to be accomplished during Day Two Laboratory.

V. ENTRY LEVEL BEHAVIOR:

A. Having performed the Day One Laboratory procedures

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be acquainted with the various areas of instructional material covered in the training course manual and better be able to utilize them in an open book laboratory situation which immediately follows the briefing session.

B. Conditions: Instructional material as covered in various parts of course training manual and as directed by verbal instructions.

C. Accepted Performance: Utilization of assigned pages of course training manual in conjunction with notes taken during briefing sessions.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training Manual Outlines:

Outline	Section	Purpose
MPN Methods	48 hour examination	Provides correct technique of culture transfer and approved methods
Verified MF Tests	Verification of Fecal Coliforms	Continuation of verification procedures
Detailed Membrane Filter Methods	Tests for Coliform Group	Provides procedural information

2. Suggested Media:

a. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review agenda for time sequencing and written material for content and prepare briefing.

2. Prepare handout (or use blackboard, if desired) indicating to trainee the written material to review. An example of such a handout would be as follows:

Item	Outline and Pages	Standard Methods (14th Edition)
MPN Methods 48 hour Procedures)	Examination of Water for Coliforms and Streptococcal Groups 3-13; 3-15; 3-15 to 3-17; 3-21; 3-24 3-20	916 to 917; 920 to 921 943; 922
MF Methods	Detailed Membrane Filter Methods 11-5 (D.7) to 11-6 (D.16)	932 to 933

B. Sequencing:

1. MF Methods

2. MPN Methods (48 hour test procedures)

3. Continue with MPN test rack (the 48 hour examination for Coliforms and Fecal Streptococci) as demonstration.

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom Equipment and Supplies:

1. Blackboard and chalk.

2. Demonstration setup.

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS:

A. None required

MPN Test Procedure

48 Hour Examination.

Read and Record all tube results. Transfer all positive lactose tubes to BGB and EC. EC tubes are discarded after 24 hours. All negative lactose tubes are discarded after 48 hours. All EVA tubes which are negative have to be inoculated again from its original postive AD tube which was retained from Monday.

MF Test Procedure

Run a given sample using 5 given sample volumes using the m-ENDO broth plates which were prepared Monday. Selected sample volumes will depend on particular sample utilized.

Sample Volumes: (Example)

25ml ; 15ml ; 8ml ; 3ml ; .8ml

MF Verification Test

Continue verification test.

Inoculate positive lactose tubes to EC tubes and place in 44.5C incubator. Reincubate negative lactose tubes for an additional 24 hours. Record Results.

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Use of Tables of MPN

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 45 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: Knowledge of this material will enable the participant to process completed laboratory MPN data sheets to compute the count/100 ml.

V. ENTRY LEVEL BEHAVIOR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be able to properly utilize the MPN Tables to obtain MPN counts based upon laboratory data sheet recordings of tube positive and negative results.

B. Conditions: Instructional material as covered in course training manual and the availability of the current edition of Standard Methods for the Examination of Water and Wastewater for reference.

C. Accepted Performance: In attendance to the lecture covering subject material. Course examination may contain questions regarding this subject.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training manual outline: "Use of Tables of Most Probable Numbers". Part 1

2. Standard Methods for the Examination of Water and Wastewater (for the 14th Edition: Pgs. 923-926).

3. Eight simulated data sheets as handouts to each participant to be used as group instructional device.

B. Suggested Media:

1. None.

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review training manual outline and pertinent pages of Standard Methods.

2. Review the eight handout data sheets for proper responses.

3. Prepare lesson.

B. Sequencing:

1. A convenient sequencing for presentation of this subject material is that which follows from the training manual outline (Part 1). This will also allow the participants to raise questions with the sequential progression through the pages of the outline which should have been read by the participants.

2. Handout the simulated 8 data sheets to each participant and as a group exercise obtain the correct MPN values.

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. For each student:

1. Classroom equipment and supplies.

2. Blackboard and chalk.

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS:

A. Eight handouts (Instructors solutions given):

No.	Data Sheet Identification	Fecal ECB Code	Code Selected	MPN per 100 ml*
1	52	5-2-1-0	5-2-1	700
2	53	1-0-0-0	1-0-0	20
3	54	5-4-1-0	5-4-1	1700
4	55	5-4-2-1	5-4-3	2800
5	56	0-0-0-0	0-0-0	<20
6	57	0-1-0-0	0-1-0	200
7	58	5-5-1-0	5-1-0	3300
8	59	X-X-5-5	5-5-5	≥24000

*14th Edition Standard Methods

FECAL COLIFORM TEST

Multiple Dilution Tube (MPN) Method

Sample Source Effluent - Plant 2 Lab. No. 52

Station 6 Collection Date 1/12/74 Time 10:00 A/P M

Received in Laboratory 10:30 A/P M Test Started 10:30 A/P M

By H L Jeter

Remarks

ml sample per tube	tube code	Presumptive LLSTB 24 hr.	Presumptive LLSTB 48 hr.	Fecal ECB 24 hr.	No. Tubes Positive
1.0	a	+		+	
	b	+		+	
	c	+		+	
	d	+		+	
	e	+		+	
0.1	a	+		+	
	b	+		+	
	c	−			
	d	−			
	e	+		−	
0.01	1a	−			
	1b	−			
	1c	+		+	
	1d	−	+	−	
	1e	−	−		
0.001	2a	−	−		
	2b	−	−		
	2c	−	−		
	2d	−	−		
	2e	−	−		

Code of Positive Tubes: - - MPN Index: _____

Fecal Coliforms: ____/100 ml Reported by: _____

17-3

FECAL COLIFORM TEST

Multiple Dilution Tube (MPN) Method

Sample Source Effluent - Plant 4 Lab. No. 53

Station 4 Collection Date 1/12/94 Time 9:45 A/P/M

Received in Laboratory 10:20 A/P/M Test Started 10:30 A/P/M

By N L J

Remarks

ml sample per tube	tube code	Presumptive LLSTB 24 hr.	Presumptive LLSTB 48 hr.	Fecal ECB 24 hr.	No. Tubes Positive
1.0	a	-	+	-	
	b	-	-		
	c	+		+	
	d	-	+	-	
	e	-	-		
0.1	a	-	+	-	
	b	-	-		
	c	-	-		
	d	-	-		
	e	-	-		
0.01	1a	-	-		
	1b	-	-		
	1c	-	-		
	1d	-	-		
	1e	-	-		
0.001	2a	-	-		
	2b	-	-		
	2c	-	-		
	2d	-	-		
	2e	-	-		

Code of Positive Tubes: - - MPN Index ____

Fecal Coliforms: ____/100 ml Reported by: ____

17-4

FECAL COLIFORM TEST

Multiple Dilution Tube (MPN) Method

Sample Source Effluent - Plant 16 Lab. No. 54

Station 3 Collection Date 1/12/74 Time 9:30 A/P M

Received in Laboratory 10:20 A/P M Test Started 10:30 A/P M

By N L J

Remarks

ml sample per tube	tube code	Presumptive LLSTB 24 hr.	Presumptive LLSTB 48 hr.	Fecal ECB 24 hr.	No. Tubes Positive
1.0	a	+		+	
	b	+		+	
	c	+		+	
	d	+		+	
	e	+		+	
0.1	a	+		+	
	b	+		+	
	c	+		+	
	d	+		-	
	e	+		+	
0.01	1a	+			
	1b	-	+	-	
	1c	-	+	-	
	1d	+		+	
	1e	-	-		
0.001	2a	-	-		
	2b	-	-		
	2c	-	-		
	2d	-	-		
	2e	-	-		

Code of Positive Tubes: - - MPN Index ____

Fecal Coliforms: ____/100 ml Reported by: ____

17-5

FECAL COLIFORM TEST

Multiple Dilution Tube (MPN) Method

Sample Source Effluent - Plant 22 Lab. No. 55

Station 4 Collection Date 1/12/74 Time 9:15 A/M

Received in Laboratory 10:20 A/M Test Started 10:30 A/M

By H L J

Remarks

ml sample per tube	tube code	Presumptive LLSTB 24 hr.	Presumptive LLSTB 48 hr.	Fecal ECB 24 hr.	No. Tubes Positive
1.0	a	+		+	
	b	+		+	
	c	+		+	
	d	+		+	
	e	+		+	
0.1	a	+		+	
	b	+		+	
	c	+		+	
	d	−	−		
	e	+		+	
0.01	1a	+		+	
	1b	+		−	
	1c	+		+	
	1d	−	−		
	1e	−	+	−	
0.001	2a	−			
	2b	−	+	+	
	2c	+			
	2d	−	+	−	
	2e	+		−	

Code of Positive Tubes: - - MPN Index ____

Fecal Coliforms: ____/100 ml Reported by: ____

FECAL COLIFORM TEST

Multiple Dilution Tube (MPN) Method

Sample Source Effluent - Plant 2 Lab. No. 56

Station 6 Collection Date 1/11/74 Time 9:40 AM/PM

Received in Laboratory 11:00 AM/PM Test Started 11:05 AM/PM

By H L J

Remarks

ml sample per tube	tube code	Presumptive LLSTB 24 hr.	Presumptive LLSTB 48 hr.	Fecal ECB 24 hr.	No. Tubes Positive
1.0	a	-	-		
	b	-	+	-	
	c	-	+	-	
	d	-	-		
	e	-	+	-	
0.1	a	-	-		
	b	-	-		
	c	-	+		
	d	-	-		
	e	-	-		
0.01	1a	-	-		
	1b	-	-		
	1c	-	-		
	1d	-	-		
	1e	-	-		
0.001	2a	-	-		
	2b	-	-		
	2c	-	-		
	2d	-	-		
	2e	-	-		

Code of Positive Tubes: - - MPN Index: _____

Fecal Coliforms: ____/100 ml Reported by: _____

17-7

FECAL COLIFORM TEST

Multiple Dilution Tube (MPN) Method

Sample Source Effluent - Plant 4 Lab. No. 57

Station 4 Collection Date 1/17/74 Time 9:50 AM

Received in Laboratory 11:00 AM Test Started 11:10 AM

By JLJ

Remarks

ml sample per tube	tube code	Presumptive LLSTB 24 hr.	Presumptive LLSTB 48 hr.	Fecal ECB 24 hr.	No. Tubes Positive
1.0	a	+		-	
	b	-	+	-	
	c	+		-	
	d	-	-		
	e	-	-		
0.1	a	-	-		
	b	+		+	
	c	-	-		
	d	-	+	-	
	e	-	-		
0.01	1a	-	-		
	1b	-	-		
	1c	-	-		
	1d	-	-		
	1e	-	-		
0.001	2a	-	-		
	2b	-	-		
	2c	-	-		
	2d	-	-		
	2e	-	-		

Code of Positive Tubes: - - MPN Index ____

Fecal Coliforms: ____/100 ml Reported by: ____

17-8

FECAL COLIFORM TEST

Multiple Dilution Tube (MPN) Method

Sample Source Effluent - Plant 1L Lab. No. 58

Station 3 Collection Date 1/14/74 Time 10:20 A/P M

Received in Laboratory 11:00 A/P M Test Started 11:15 A/P M

By JJ LJ

Remarks

ml sample per tube	tube code	Presumptive LLSTB 24 hr.	Presumptive LLSTB 48 hr.	Fecal ECB 24 hr.	No. Tubes Positive
1.0	a	+		+	
	b	+		+	
	c	+		+	
	d	+		+	
	e	+		+	
0.1	a	+		+	
	b	+		+	
	c	+		+	
	d	+		+	
	e	+		+	
0.01	1a	−	+	−	
	1b	−	−		
	1c	+		+	
	1d	−	−		
	1e	−	−		
0.001	2a	−	+	−	
	2b	−	−		
	2c	−	+	−	
	2d	−	−		
	2e	−	−		

Code of Positive Tubes: - - MPN Index ____

Fecal Coliforms: ____/100 ml Reported by: ____

17-9

FECAL COLIFORM TEST

Multiple Dilution Tube (MPN) Method

Sample Source Effluent - Plant 22 Lab. No. 59

Station 4 Collection Date 1/14/74 Time 10:00 AM

Received in Laboratory 11:00 AM Test Started 11:20 AM

By _____

Remarks

ml sample per tube	tube code	Presumptive LLSTB 24 hr.	Presumptive LLSTB 48 hr.	Fecal ECB 24 hr.	No. Tubes Positive
1.0	a	+			
	b	+			
	c	+			
	d	+			
	e	+			
0.1	a	+			
	b	+			
	c	+			
	d	+			
	e	+			
0.01	1a	+		+	
	1b	+		+	
	1c	+		+	
	1d	+		+	
	1e	+		+	
0.001	2a	+		+	
	2b	+		+	
	2c	+		+	
	2d	+		+	
	2e	+		+	

Code of Positive Tubes: - - MPN Index _____

Fecal Coliforms: ____/100 ml Reported by: _____

17-10

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Verified Membrane Filter Tests

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 40 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: Participant will be aware of need and technique of performing the verification test for each of the standard indicator bacteria.

V. ENTRY LEVEL BEHAVIOR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be able to utilize standard or experimental membrane filter plates, intended for Total Coliform, fecal Coliform, and fecal Streptococcus, to obtain pure cultures and process them for identification and calculation of a percent verification.

B. Conditions: Instructional material as covered in course training manual and the availability of the current edition of Standard Methods for the Examination of Water and Wastewater for reference. Verification test has already been initiated for the fecal coliforms during the first laboratory session allowing the participant some prior knowledge of this subject.

C. Accepted Performance: In attendance to the lecture covering subject material. Course examination may contain questions regarding this subject.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training manual outline: "Verified Membrane Filter Tests"

2. Standard Methods for the Examination of Water and Wastewater (for the 14th Edition: Pgs. 931; 935; 945)

B. Suggested Media:

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review training manual outline and pertinent pages of Standard Methods.

2. Prepare lesson.

B. Sequencing:

1. A convenient sequencing for presentation of this subject material is that which follows from the training manual outline. This will also allow the participants to raise questions as they not only should have read the outline but have processed fecal Coliform cultures for verification in the laboratory exercises.

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. For each student:

1. Classroom equipment and supplies.

2. Blackboard and chalk.

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS:

A. None required.

Instructor may elect to show laboratory preparations of the verification test during the presentation. Since the fecal Coliforms were participant processed, the demonstration choice could involve the Total Coliform and/or fecal Streptococci.

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Bacteriological Laboratory: Equipment, Materials, and Supplies

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 1 hour and 10 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: Participant will be aware of the materials, equipment, and supplies that should be available to the bacteriological laboratory to perform in an acceptable manner.

V. ENTRY LEVEL BEHAVIOR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be knowledgeable of the materials, supplies, and equipment which should be available and operational in a bacteriological laboratory which is capable, dependent upon correct analytical procedures, of performing in an acceptable manner.

B. Conditions: Instructional handout material (if desirable by instructor) listing Capital Equipment, Reusable supplies, and consumable supplies of some representative test procedures by a bacteriological laboratory.

Current Edition of Standard Methods for the Examination of Water and Wastewater for reference.

Some exposure to bacteriological laboratory material, equipment and supplies has already occurred during laboratory sessions.

C. Accepted Performance: In attendence to the lecture covering subject material and instructor observation of techniques during laboratory sessions. Course examination may contain questions regarding this subject.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Standard Methods for the Examination of Water and Wastewater (for the 14th Edition: Pgs. 880-884, 885-886, 887-891).

2. Equipment and Supply Requirements (Instructor Guides and/or handout material)-Representative Procedures.

a. Standard Plate Count (follows this IPW)

b. Coliform Test by the Multiple Dilution Tube (MPN) Method

c. Completed Test for the MPN Method

d. Total Coliform Test by the Membrane Filter Method

3. Training Manual Outline: Testing The Suitability of Distilled Water for the Bacteriology Laboratory.

4. Instructor Reference: Laboratory Safety Practices.

B. Suggested Media:

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review written materials.

Material	Remarks
Standard Methods	Equipment, material, and supply requirements.
Equipment and Supply Representative-Procedures	Review for typical requirements of a bacteriological laboratory-could be helpful to participant as a handout.
Outline: Testing the Suitability of Distilled Water for the Bacteriological Laboratory	Describes test procedure and requirements of a suitable water for laboratory use.
Reference: Laboratory Safety Practices	General laboratory safety

2. Prepare lesson.

B. Sequencing:

1. A convenient approach to this subject would be to:

a. Discuss the need for, and basically cover, the distilled water subject.

b. Utilize the equipment and supply requirement lists to describe a suitably equipped laboratory. Indicate unsuitable practices as the subject occurs such as:

Accepted Practice	Unsuitable Practice
Autoclave meeting requirements	Poor pressure cooker equipment

pH meter meeting requirements	Poor practice of using paper pH indicator
incubator meeting requirements	Incubator with "hot" and "cold" spots which are used.
Refrigerator meeting requirements	Refrigerator freezing contents; having edible foods; containing organic material fumes, etc.

c. Discuss General Laboratory Safety.

d. If time and facility arrangement allows, a tour through laboratory preparation area could be scheduled.

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom equipment and supplies.

1. None

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS:

A. None required.

WATER MONITORING PROCEDURE: Standard Plate Count

Equipment and Supply Requirements

A. Capital Equipment:

1. Autoclave*, providing uniform temperatures up to and including 121° C, equipped with an accurate thermometer, pressure gauges, saturated steam power lines and capable of reaching required temperature within 30 minutes.
2. Balance, 0.1 g sensitivity at load of 150 g.
3. Incubator*, air, to operate at 35° C ± 0.5° C.
4. Oven*, hot-air sterilizing, to give uniform temperatures and with suitable thermometer to register accurately in range of 160-180° C.
5. pH Meter, accurate to at least 0.1 pH unit, with standard pH reference solution(s).
6. Water Distillation Apparatus*, glass or block tin, or source of distilled water suitable for bacteriological operations.
7. Incubator*, water, to operate at 45° ± 1° C.
8. Refrigerator*, to operate at 4° C.
9. Thermometer, mercury bulb, certified NBS or calibrated against a certified NBS thermometer 0.5° intervals and have 160-180° C as part of range.

B. Reusable Supplies:

1. Apron or coat suitable for laboratory.
2. Baskets, wire for discarded cultures.
3. Bottles, dilution*, 6 oz. screw caps, with 99 ml volume level etched on one side.
4. Bottles, sample*, preferred characteristics being 250 ml (6-8 oz.), wide mouth, glass stopper.
5. Bottle, squeeze type, with disinfecting solution.
6. Burner, gas, Bunsen burner type.
7. Cans, pipet, aluminum or steel; not copper (If plastic, or other type of prepackaged disposable pipets are used, this item is unnecessary.)
8. Counter, colony, Quebec type, Darkfield model with guide plate, hand talley.
9. Cylinder, graduated, 100 ml.
10. Cylinder, graduated, 500 ml.
11. Dish*, petri, sterile, 100 mm diameter, ≥ 15 mm in height, with glass or porous tops preferred (presterilized, sterile one-time-use plastic tubes may be used).
12. Flask*, Erlenmeyer, 250 ml capacity.
13. Flask, Volumetric, 1 liter.
14. Pan, to receive discarded contaminated pipets and glassware (must contain disinfectant before use).
15. Pipets*, 1 ml, having 0.1 ml increments, sterile, cotton plugged, glass or disposable plastic, TD type (NOT a "blowout" type).
16. Pipets, 5 ml, having 1 ml increments (have several on hand).
17. Sponge, for cleaning desk top.
18. Thermometer, mercury bulb, certified NBS or calibrated against a certified NBS thermometer 0.5° intervals and have 30-40° C as part of range.

WATER MONITORING PROCEDURE: Standard Plate Count

Equipment and Supply Requirements (Continued)

C. Supplies Used Up in the Analysis (must be replaced when stocks get low):

1. Cotton, nonabsorbent.
2. Disinfectant, for bench tops. (Use household bleach solution prepared according to instructions on bottle.)
3. Distilled water, suitable for bacteriological cultures (note distillation apparatus required in capital equipment).
4. EDTA (ethylene dinitrilotetraacetic acid).
5. Foil, aluminum.
6. Paper, Kraft.
7. Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$) (recommend purchase of 1/4 lb. units).
8. Pencil, wax, (recommend soft wax equivalent to Blaisdell 169T).
9. Potassium Dihydrogen Phosphate (KH_2PO_4) (recommend purchase of 1/4 lb. units).
10. Sheet, Data, SPC.
11. Sodium Hydroxide (NaOH).
12. Sodium Thiosulfate ($Na_2S_2O_3.5\ H_2O$).
13. Tryptose Glucose Yeast Agar, dehydrated medium (recommend purchase of 1/4 lb. unit).

*Items marked are needed in quantities or require size or space allowances which cannot be specified here, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specifications for equipment of supplies, see the Microbiology Section of the current edition of Standard Methods for the Examination of Water and Wastewater.

WATER MONITORING PROCEDURE: Coliform Test by the Multiple Dilution Tube (MPN) Method

Equipment and Supply Requirements

A. Capital Equipment:

1. Autoclave, providing uniform temperatures up to and including 121° C, equipped with an accurate thermometer, pressure gauges, saturated steam power lines and capable of reaching required temperature within 30 minutes
2. Balance, 0.1 g sensitivity at load of 150 g
3. Air Incubator to operator at 35° C ± 0.5° C
4. Oven, *hot-air sterilizing, to give uniform temperatures and with suitable thermometer to register accurately in range of 160-180° C
5. pH Meter, accurate to at least 0.1 pH unit, with standard pH reference solutions(s)
6. Water distillation apparatus, (glass or block tin), or source of distilled water suitable for bacteriological operations

B. Reusable Supplies:

1. Apron or coat suitable for laboratory
2. Baskets, wire for discarded cultures
3. Bottles, dilution*, 6-oz. screw caps, with 99 ml volume level etched on one side
4. Bottles, sample*, preferred characteristics being 250 ml (6-8 oz.), wide mouth, glass stopper
5. Burner, gas, Bunsen burner type
6. Cans, pipet, aluminum or steel; not copper (If plastic, or other type of prepackaged disposable pipets are used, this item is unnecessary.)
7. Metal caps* to fit 18 and 25 mm culture tubes
8. Pan, to receive discarded contaminated pipets and glassware (must contain disinfectant before use)
9. Inoculation loop, 3 mm diameter loop of nichrome or platinum-iridium wire, 26 B&S gauge, in holder
10. Pipets*, 1 ml, with 0.1 ml graduations, Mohr type preferred, sterile, cotton plugged, glass or disposable plastic
11. Pipets*, 10 ml, with 1.0 ml graduations, Mohr type preferred, sterile, cotton plugged, glass or disposable plastic
12. Racks, culture type*, 10 x 5 openings, to accept tubes at least 25 mm in diameter
13. Sponge, for cleaning desk top
14. Tubes, culture*, 150 x 25 mm
15. Tubes, culture*, 150 x 18 mm
16. Tubes, fermentation*, 75 x 10 mm vials to be inverted in culture tubes

C. Consumable Supplies:

1. Distilled water, suitable for bacteriological cultures (note distillation apparatus required in capital equipment)
2. BGLBB (Brilliant Green Lactose Bile Broth), dehydrated (recommend purchase of 1/4 lb. units)
3. Lactose Lauryl Sulfate Tryptose Broth, dehydrated (recommend purchase of 1 lb. units)
4. Postasium Dihydrogen Phosphate (KH_2PO_4) (recommend purchase of 1/4 lb. units)

WATER MONITORING PROCEDURE: Coliform Test by the Multiple Dilution Tube (MPN) Method

C. Consumable Supplies (Continued):

5. Disinfectant, for bench tops. (Use household bleach solution prepared according to instructions on bottle)
6. Wax pencils (recommend soft wax equivalent to Blaisdell 169T)
7. EDTA (ethylene dinitrilotetraacetic acid)
8. Sodium thiosulfate ($Na_2S_2O_3.5\ H_2O$)

*Items marked are needed in quantities or require size or space allowances which cannot be specified here, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specifications for equipment and supplies, see the Microbiology Section of the current edition of "Standard Methods for the Examination of Water and Wastewater."

WATER MONITORING PROCEDURE: Completed Test for the MPN Method

Equipment and Supply Requirements

A. Capital Equipment:

1. *Incubator, air, to operate at 35° ± 0.5°C
2. *Oven, hot air, sterilizing-drying, to give uniform temperatures and with suitable thermometer to register accurately in range of 160-180°C
3. *Autoclave, providing uniform temperatures up to and including 121°C, equipped with an accurate thermometer, pressure gauges, saturated steam power lines and capable of reaching required temperature within 30 minutes
4. Balance, 0.1 g sensitivity at load of 150 g
5. pH Meter, accurate to at least 0.1 pH unit, with standard pH reference solution(s)
6. Water distillation apparatus, (glass or block tin), or source of distilled water suitable for bacteriological operations
7. Microscope, compound, oil immersion lens, Abbé condenser

B. Reusable Supplies:

1. Apron or coat suitable for laboratory
2. Baskets, wire for discarded cultures
3. Tubes, culture*, 150 x 18 mm (metal caps for fermentation and screw-cap for slants)
4. Tubes, fermentation*, 75 x 10 mm vials to be inverted in culture tubes
5. Inoculation loop and needle, 3 mm diameter for loop and both of nichrome or platinum-iridium wire, 26 B&S gauge, in holders
6. Hotplate with magnetic whirl feature, if desired
7. Burner, gas, Bunsen burner type
8. Sponge, for cleaning desk top
9. Counter, colony, Quebec type, Darkfield Model with guide plate
10. Racks, culture type*, 10 x 5 openings, to accept tubes at least 25 mm in diameter
11. Pan, to receive discarded contaminated pipets and glassware (must contain disinfectant before use)
12. *Flasks, Erlenmeyer, 500 ml; 300 ml; 250 ml
13. *Cylinder, 500 ml; 250 ml

C. Consumable Supplies:

1. Bibulous paper
2. Dishes, petri, 100 x 15 mm sterile plastic, disposable
3. Disinfectant, for bench tops. (Can use household bleach solution prepared according to instructions on bottle.)
4. Distilled water, suitable for bacteriological cultures (Note distillation apparatus required in capital equipment.)
5. Eosin methylene blue agar, dehydrated (Levine modification)
6. Gram stain solutions, complete set
7. Lactose Lauryl Sulfate Tyrptose Broth, dehydrated
8. Nutrient agar, dehydrated
9. Slides, microscopic, glass 1" x 3"

10. Foil, aluminum
11. Matches or striker
12. Wax pencils (recommend soft as equivalent to Blaisdell 169T)

*Items marked are needed in quantities or require size or space allowances which cannot be specified here, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specifications for equipment and supplies, see the Microbiology Section of the current edition of "Standard Methods for the Examination of Water and Wastewater."

WATER MONITORING PROCEDURE: Total Coliform Test by the Membrane Filter Method

Equipment and Supply Requirements

A. Capital Equipment:

1. Autoclave, steam, providing uniform temperatures up to and including 121°C and equipped with an accurate thermometer, pressure gauges, saturated steam power lines and capable of reaching required temperatures within 30 minutes. (Alternately, a suitable pressure cooker is acceptable--see Standard Methods for particulars.)
2. Incubator, air, providing uniform and constant temperature of 35°C. +0.5°C and having an atmosphere of at least 90% relative humidity.
3. Oven, hot-air, providing uniform temperatures within the range of 160-180°C
4. Apparatus, water distillation, distilled water product suitable for bacteriological operations (alternately, a suitable source is permissible).
5. Microscope, stereoscopic, 10X to 15X magnification with fluorescent lighting mandatory. (Alternately, a suitable magnifying lens with fluorescent lamp is acceptable.)
6. Refrigerator, set for less than 10°C but above the freezing temperature.
7. Vacuum source, preferably a pump assembly with suitable hoses and shut-off valve provided. (Alternately, an aspirator or hand pump with the same provisions are acceptable.)
8. Balance, analytical, sensitivity of 1 mg.
9. Gas source, suitable for burner. (Alternately, an alcohol lamp can be used.)

B. Reusable Supplies:

1. Apron, suitable for laboratory operations.
2. Bottle, sample, of sufficient size for standard sample, preferably of 250 ml, wide-mouth, glass stopper, with tag. (Alternately, 120 ml size)
3. Bottle, squeeze type, containing disinfecting solution.
4. Burner, gas, suitable for laboratory operations with connecting hose.
5. Thermometer, NBS (or NBS calibrated), functions within 20°-60°C range with individual markings of 1°C.
6. Thermometer, NBS (or NBS calibrated), functions within 150°-190°C range with individual markings of 1°C.
7. Filtration Unit, MF, a seamless funnel attached to a receptacle bearing a porous plate (screen, porous disc, etc.) and constructed from stainless steel, glass, porcelain, plastic, or other suitable material.
8. Hot plate, controllable heat range up to the 100°C range.
9. Balance, trip, sensitivity of 0.1 gram at a load of 150 grams, with appropriate weights.
10. Meter, pH, accurate to within 0.1 pH unit, with suitable standard pH reference solution(s).
11. Can, pipet, non-toxic and sterilizable material (if pre-sterilized disposable type pipets are used, this item is unnecessary).
12. Pan, discard, receives contaminated material and pipets and contains disinfectant. Should be of sufficient length to receive pipets placed horizontally.
13. Cylinder, graduated, 500 ml, 100 ml, 50 ml. and 25 ml size. (The 50 ml size is covered with a "cap" of foil or Kraft paper and then sterilized.)

Equipment and Supply Requirements (Continued)

14. Blank, dilution water, 99 ml.
15. Pipets, microbiological, 50. ml, with 0.1 ml graduations, sterile cotton plugged, glass or disposable types (the disposable types are for one time use and may be glass or plastic).
16. Pipets, microbiological, 1.0 ml, with 0.1 graduations, sterile cotton plugged, glass or disposable types (the disposable types are for one time use and may be glass or plastic).
17. Pipets, microbiological, 10 ml, with 1 ml graduations, sterile, cotton plugged, glass or disposable types (the disposable types are for one-time use and may be glass or plastic).
18. Beaker, 50 ml (for measuring pH).
19. Flask, volumetric, 1 liter capacity (for stock solution of phosphate buffer).
20. Flask, Erlenmeyer, 500 ml capacity (for holding buffered distilled rinse water).
21. Flask, sidearm, 1 liter size (for reservoir of MF apparatus; proper size bored, rubber stopper is needed to connect MF filtration flask to flask and hose required to vacuum source (must be rigid enough to avoid collapse under vacuum and flexible enough to be controlled by pinch clamp) pinch clamp - vacuum control.
22. Flask, Erlenmeyer, 50 ml (for preparing m-ENDO medium).
23. Forceps, curved end, round tip.
24. Bottle, small, Methanol or Ethanol volume to cover ends of forceps.
25. Sponge, small, to spread and wipe germicide.
26. Desiccator, media storage, ideally opaque or darkened and containing desiccating agent to remove moisture.

C. Consumable Supplies:

1. Dish, petri, disposable, tight fitting plastic, 50 x 12 mm, sterile.
2. m-ENDO Broth, medium, dehydrated, total coliform. Distributors, Difco, BBL, or other equivalent preparation.
3. Pencil, wax, recommended of soft wax equivalent to Blaisdell 169T.
4. Tags, bottle marking.
5. Glass Wool.
6. Cotton, non-absorbent.
7. Paper, Kraft wrapping.
8. Foil, aluminum, heavy duty.
9. Matches or striker.
10. Towels, paper.
11. Detergent, non-toxic, laboratory cleaning.
12. Data Sheet, as required by analyst's agency.
13. Filter, membrane, 47mm, 0.45 µm pore size, white, grid marked, sterile.
14. Pad, absorbent, 48 mm, sterile (usually included with membrane packet).
15. Potassium Dihydrogen Phosphate (KH_2PO_4), recommended 1/4 lb.
16. Sodium Thiosulphate ($Na_2S_2O_3SH_2O$).
17. Disinfectant, for bench tops and decontaminating purposes, bleach of household strength and prepared according to label directions.
18. Sodium Hydroxide (NaOH), 1N.
19. Distilled water, suitable for bacteriological operations. Obtainable from distillation apparatus (see Capital Equipment) or suitable source of supply.

Equipment and Supply Requirements (Continued)

20. Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$).
21. Ethanol, 95%.

Item needs in quantities or required size or space allowances cannot be specified, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specifications for equipment and supplies, see the Microbiology Section of the current edition of Standard Methods for the Examination of Water and Wastewater.

LABORATORY SAFETY PRACTICES

I INTRODUCTION

A Safe Use, Handling and Storage of Chemicals

1 Chemicals in any form can be safely stored, handled, and used if their hazardous physical and chemical properties are fully understood and the necessary precautions, including the use of proper safeguards and personal protective equipment are observed.

2 The management of every unit within a manufacturing establishment must give wholehearted support to a well integrated safety policy.

B General Rules for Laboratory Safety

1 Supervisory personnel should think "safety." Their attitude toward fire and safety standard practices is reflected in the behavior of their entire staff.

2 A safety program is only as strong as the worker's will to do the correct things at the right time.

3 The fundamental weakness of most safety programs lies in too much lip service to safety rules and not enough action in putting them into practice.

4 Safety practices should be practical and enforceable.

5 Accident prevention is based on certain common standards of education, training of personnel and provision of safeguards against accidents.

II LABORATORY DESIGN AND EQUIPMENT

A Type of Construction

1 Fire-resistant or noncombustible

2 Multiple story buildings should have adequate means of exit.

3 Stairways enclosed with brick or concrete walls

4 Laboratories should have adequate exit doors to permit quick, safe escape in an emergency and to protect the occupants from fires or accidents in adjoining rooms. Each room should be checked to make sure there is no chance of a person being trapped by fire, explosions, or release of dangerous gases.

5 Laboratory rooms in which most of the work is carried out with flammable liquids or gases should be provided with explosion-venting windows.

B Arrangement of Furniture and Equipment

1 Furniture should be arranged for maximum utilization of available space and should provide working conditions that are efficient and safe.

2 Aisles between benches should be at least 4 feet wide to provide adequate room for passage of personnel and equipment.

3 Desks should be isolated from benches or adequately protected.

4 Every laboratory should have an eye-wash station and a safety shower.

C Hoods and Ventilation

1 Adequate hood facilities should be installed where work with highly toxic or highly flammable materials are used.

2 Hoods should be ventilated separately and the exhaust should be terminated at a safe distance from the building.

3 Make-up air should be supplied to rooms or to hoods to replace the quantity of air exhausted through the hoods.

4 Hood ventilation systems are best designed to have an air flow of not less than 60 linear feet per minute across the face of the hood, with all doors open and 150, if toxic materials are involved.

5 Exhaust fans should be spark-proof if exhausting flammable vapors and corrosive resistant if handling corrosive fumes.

6 Controls for all services should be located at the front of the hood and should be operable when the hood door is closed.

7 All laboratory rooms should have the air changed continuously at a rate depending on the materials being handled.

D Electrical Services

1 Electrical outlets should be placed outside of hoods to afford easy access and thus protect them from spills and corrosion by gases.

2 Noninterchangeable plugs should be provided for multiple electrical services.

3 Adequate outlets should be provided and should be of the three-pole type to provide for adequate grounding.

E Storage

1 Laboratories should provide for adequate storage space for mechanical equipment and glassware which will be used regularly.

2 Flammable solvents should not be stored in glass bottles over one liter in size. Large quantities should be stored in metal safety cans. Quantities requiring containers larger than one gallon should be stored outside the laboratory.

3 Explosion proof refrigerators should be used for the storage of highly volatile and flammable solvents.

4 Cylinders of compressed or liquified gases should not be stored in the laboratory.

F Housekeeping

1 Housekeeping plays an important role in reducing the frequency of laboratory accidents. Rooms should be kept in a neat orderly condition. Floors, shelves, and tables should be kept free from dirt and from all apparatus and chemicals not in use.

2 A cluttered laboratory is a dangerous place to work. Maintenance of a clean and orderly work space is indicative of interest, personal pride, and safety-mindedness.

3 Passageways should be kept clear to all building exits and stairways.

4 Metal containers should be provided for the disposal of broken glassware and should be properly labeled.

5 Separate approved waste disposal cans, should be provided for the disposal of waste chemicals.

6 Flammable liquids not miscible with water and corrosive materials, or compounds which are likely to give off toxic vapors should never be poured into the sink.

G Fire Protection

1 Laboratory personnel should be adequately trained regarding pertinent fire hazards associated with their work.

2 Personnel should know rules of fire prevention and methods of combating fires.

3 Fire extinguishers (CO_2 type) should be provided at convenient locations and personnel should be instructed in their use.

4 Automatic sprinkler systems are effective for the control of fires in chemical laboratories.

H Alarms

1 An approved fire alarm system should be provided.

2 Wherever a hazard of accidental release of toxic gases exists, a gas alarm system to warn occupants to evacuate the building should be provided.

3 Gas masks of oxygen or compressed air type should be located near exits and selected personnel trained to use them.

III HANDLING GLASSWARE

A Receiving, Inspection and Storage

1 Packages containing glassware should be opened and inspected for cracked or nicked pieces, pieces with flaws that may become cracked in use, and badly shaped pieces.

2 Glassware should be stored on well-lighted stockroom shelves designed and having a coping of sufficient height around the edges to prevent the pieces from falling off.

B Laboratory Practice

1 Select glassware that is designed for the type of work planned.

2 To cut glass tubing or a rod, make a straight clean cut with a cutter or file at the point where the piece is to be severed. Place a towel over the piece to protect the hands and fingers, then break away from the body.

3 Large size tubing is cut by means of a heated nichrome wire looped around the piece at the point of severance.

4 When it is necessary to insert a piece of glass tubing or a rod through a perforated rubber or cork stopper, select the correct bore so that the insertion can be made without excessive strain.

5 Use electric mantels for heating distillation apparatus, etc.

6 To remove glass splinters, use a whisk broom and a dustpan. Very small pieces can be picked up with a large piece of wet cotton.

IV GASES AND FLAMMABLE SOLVENTS

A Gas Cylinders

1 Large cylinders must be securely fastened so that they cannot be dislodged or tipped in any direction.

2 Connections, gauges, regulators or fittings used with other cylinders must not be interchanged with oxygen cylinder fittings because of the possibility of fire or explosion from a reaction between oxygen and residual oil in the fitting.

3 Return empty cylinders promptly with protective caps replaced.

B Flammable Solvents

1 Store in designated areas well ventilated.

2 Flash point of a liquid is the temperature at which it gives off vapor sufficient to form an ignitible mixture with the air near the surface of the liquid or within the vessel used.

3 Ignition temperature of a substance is the minimum temperature required to initiate or cause self-sustained combustion independently of the heating or heated element.

4 Explosive or flammable limits. For most flammable liquids, gases and solids there is a minimum concentration of vapor in air or oxygen below which propagation of flame does not occur on contact with a source of ignition. There is also a maximum proportion of vapor or gas in air above which

propagation of flame does not occur. These limit mixtures of vapor or gas with air, which if ignited will just propagate flame, are known as the "lower and higher explosive or flammable limits."

5 Explosive Range. The difference between the lower and higher explosive or flammable limits, expressed in terms of percentage of vapor or gas in air by volume is known as the "explosive range."

6 Vapor Density is the relative density of the vapor as compared with air.

7 Underwriter's Laboratories Classification is a standard classification for grading the relative hazard of the various flammable liquids. This classification is based on the following scale:

Ether Class	100
Gasoline Class.....	90 - 100
Alcohol (ethyl) Class....	60 - 70
Kerosene Class	30 - 40
Paraffin Oil Class.....	10 - 20

8 Extinguishing agents

V CHEMICAL HAZARDS

A Acids and Alkalies

1 Some of the most hazardous chemicals are the "strong" or "mineral" acids such as hydrochloric, hydrofluoric, sulfuric and nitric.

2 Organic acids are less hazardous because of their comparatively low ionization potentials. However, such acids as phenol (carbolic acid), hydrocyanic and oxalic are extremely hazardous because of their toxic properties.

3 Classification of acids

B Oxidizing Materials

1 Such oxidizing agents as chlorates, peroxides, perchlorates and perchloric acid, in contact with organic matter can cause explosions and fire.

2 They are exothermic and decompose rapidly, liberating oxygen which reacts with organic compounds.

3 Typical hazardous oxidizing agents are:

Chlorine Dioxide
Sodium Chlorate
Potassium Chromate
Chromium Trioxide
Perchloric Acid

C Explosive Power

1 Many chemicals are explosive or form compounds that are explosive and should be treated accordingly.

2 A few of the more common examples of this class of hazardous materials are:

Acetylides
Silver Fulminate
Peroxides
Peracetic Acid
Nitroglycerine
Picric Acid
Chlorine and Ethylene
Sodium Metal
Calcium Carbide

D Toxicity

1 Laboratory chemicals improperly stored or handled can cause injury to personnel by virtue of their toxicity.

2 Types of exposure. There are four types of exposure to chemicals:

a Contact with the skin and eyes

b Inhalation

c Swallowing

d Injection

VI PRECAUTIONARY MEASURES

A Clothing and Personal Protective Equipment

1 Chemical laboratories should have special protective clothing and equipment readily available for emergency use and for secondary protection of personnel working with hazardous materials.

2 Equipment should be provided for adequate:

a Eye protection

b Body protection

c Respiratory protection

d Foot protection

e Hand protection

B Bodily Injury

1 Burns, eye injuries, and poisoning are the injuries with which laboratory people must be most concerned.

2 First emphasis in the laboratory should be on preventing accidents. This means observing all recognized safe practices using necessary personal protective equipment and exercising proper control over poisonous substances at the source of exposure.

3 So that a physician can be summoned promptly, every laboratory should have posted the names, telephone numbers, and addresses of doctors to be called in an emergency requiring medical care.

REFERENCES

Guide for Safety in the Chemical Laboratory, the General Safety Committee of the Manufacturing Chemists Association, Inc., Van Nostrand, New York (1954).

This outline was prepared by Paul F. Hallbach, Chemist, National Training and Operational Technology Center, MOTD, OWPO, USEPA, Cincinnati, Ohio 45268

Descriptors: Safety, Laboratory, Practices Safety, Laboratory Design Chemical Storage, Gas Cylinders, Flammable Solvents

TESTING THE SUITABILITY OF DISTILLED WATER FOR THE BACTERIOLOGY LABORATORY

I INTRODUCTION

A Standard Methods for the Examination of Water and Wastewater (12th Edition) states;

"Only distilled water or demineralized water which has been tested and found free from traces of dissolved metals and bactericidal and inhibitory compounds may be used for the preparation of culture media and reagents. Bactericidal compounds may be measured by a biologic test procedure ...." This outline describes a suitable procedure.

B A need for such a test has been shown in the lack of reproducibility of plate counts and a possible cause of inconsistent results in split sample examinations.

II THEORY OF THE TEST PROCEDURE

A Growth of Aerobacter aerogenes in a chemically defined minimal growth medium. The addition of a toxic agent or a growth promoting substance will alter the 24 hr population by an increase or decrease of 20% or more, when compared to a control.

III APPARATUS AND MATERIALS

A Glassware - rinse all glassware in freshly redistilled water from a glass still. The sensitivity of the test depends upon the cleanliness of the sample containers, flasks, tubes, and pipettes. Use only borosilicate glassware.

B Culture - any strain of coliform IMViC type --++ (A. aerogenes). This can be easily obtained from any polluted river or sewage sample.

IV REAGENTS

A Use reagents of the highest purity. Some brands of potassium dihydrogen phosphate (KH_2PO_4) have large amounts of impurities. The sensitivity of the test is controlled in part by the purity of the reagents employed.

1 Carbon source - Sodium citrate, reagent, crystals ($Na_3C_6H_5O_7 \cdot 2H_2O$) 0.29 g dissolved in 500 ml of redistilled water.

2 Nitrogen source - Dissolve 0.60 g of ammonium sulfate, reagent, crystals, $(NH_4)_2SO_4$) in 500 ml of redistilled water.

3 Salt mixture solution - Dissolve the following compounds in 500 ml of redistilled water.

Magnesium sulfate, reagent, crystals ($MgSO_4 \cdot 7H_2O$) 0.26 g.

Calcium chloride, reagent, crystals ($CaCl_2 \cdot 2H_2O$) 0.17 g.

Ferrous sulfate, reagent, crystals ($FeSO_4 \cdot H_2O$) 0.23 g.

Sodium chloride, reagent, crystals (NaCl) 2.50 g.

4 Phosphate buffer solution - Use a 1 to 25 dilution of a stock phosphate solution prepared by dissolving 34.0 gm of potassium dihydrogen phosphate (KH_2PO_4) in 500 ml of distilled water, adjusting to pH 7.2 with 1 N NaOH and diluting to 1 liter with distilled water.

5 Toxic control - dissolve 0.40 grams $CuSO_4 \cdot 5H_2O$ in 100 ml of redistilled water. Dilute 1:1000 for 1 mg per liter Cu before use.

B Sterilization of Reagents

Unknown distilled water sample - either boil for one minute or sterilize by membrane filtration.

Prepare reagents with redistilled water heated to boiling for 1 to 2 minutes. Phosphate buffer solution may be sterilized by MF filtration or boiling.

C Solutions are useful up to two weeks when stored at 5°C in sterilized glass stoppered bottles. The salts solution must be stored in the dark because sunlight results in copious ferric ion precipitation. A slight turbidity arising in the first 3 - 5 days does not detract from the usefulness of the reagents.

V PROCEDURE

A Collect 150 - 200 ml of water sample in a sterile borosilicate glass flask and sterilize. Label 3 flasks or tubes: A, B, and F. Add water Samples and redistilled water to each flask as indicated at the bottom of the page.

B Add a suspension of Aerobacter aerogenes (IMViC type --++) of such density that each flask will contain 25 - 75 cells per ml. Make an initial bacterial count by plating a 1 ml sample in plate count agar. Incubate tests A-F at 32° or 35°C for 20 - 24 hr. Make plate counts using dilutions of 1, 0.1, 0.01, 0.001 and 0.0001 ml.

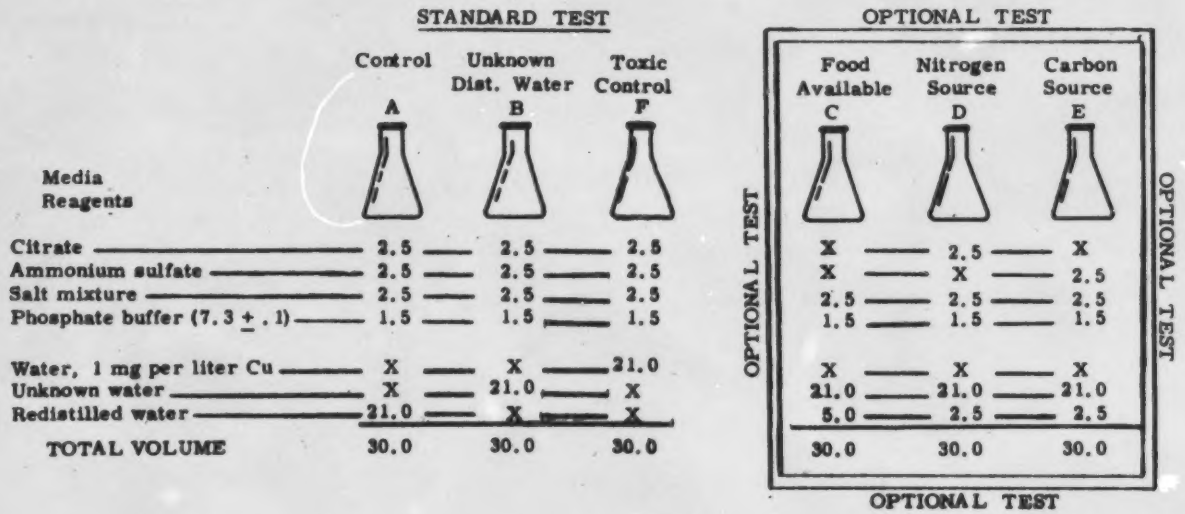
VI PREPARATION OF A BACTERIAL SUSPENSION

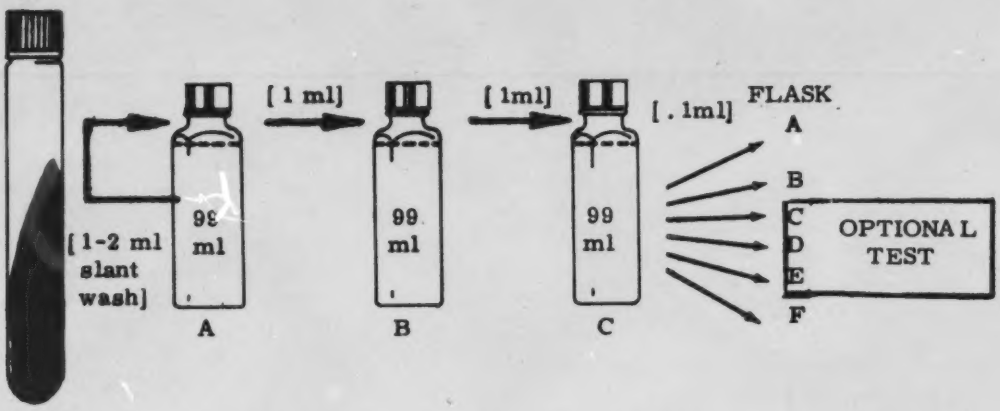
A Bacterial Growth

On the day prior to performing the distilled water suitability test, inoculate a strain of Aerobacter aerogenes onto a nutrient agar slant with a slope of approximately 2 - 1/2 inches in length contained in a 125 mm × 16 mm screw cap tube. Streak the entire agar surface to develop a continuous growth film and incubate 18 - 24 hrs at 35°C.

B Harvesting Viable Cells

Pipette 1 - 2 ml of sterile dilution water from a 99 ml water blank onto the 18 - 24 hr culture. Emulsify the growth on the





slant by gently rubbing the bacterial film with the pipette, being careful not to tear the agar, and pour the contents back into the original 99 ml water blank.

C Dilution of Bacterial Suspension

Make a 1 - 100 dilution of the original bottle into a second water blank, and a further 1 - 100 dilution of the second bottle into a third water blank, shaking vigorously after each transfer. Then pipette 0.1 ml of the third dilution (1:1, 000, 000) into each of the flasks A, B, and F (see Standard Methods for Examination of Dairy Products, 12th ed.). This procedure should result in a final dilution of the organisms to a range of 25-75 viable cells for each ml of test solution.

D Verification of Bacterial Density

Variations among strains of the same organism, different organisms, media, and surface area of agar slopes will possibly necessitate adjustment of the dilution procedure to arrive at a specific density range between 25 - 75 viable cells. To establish the growth range numerically for a specific organism and medium, make a series of plate counts from the third dilution to determine the bacterial density. Then choose the proper volume from this third dilution which when diluted by the 30 ml in the flasks A, B, and F will contain 25 - 75 viable cells per ml. If the procedures are standardized as to surface area of the slant and laboratory technique, it is possible to reproduce results on repeated experiments with the same strain of microorganisms.

E Procedural Difficulties:

1 Chlorine or chloramine distilling over into receiver. Distilled water should be checked by a suitable quantitative procedure like the starch-iodide titration. If chlorine is found, sufficient sodium thiosulfate or sodium sulfite must be added.

2 Unknown water sample stored in soft glass containers or glass containers without liners for metal caps.

3 Contamination of reagents of distilled water with a bacterial background.

4 Incorrect dilution of A. aerogenes to get 25 - 75 cells per ml.

5 Gross contamination of the sample determined by the initial colony count before incubation.

F Calculation:

1 For growth inhibiting substances:

$$\frac{\text{colony count per ml Flask B}}{\text{colony count per ml Flask A}}$$

a Ratio 0.8 to 1.2 (inclusive) shows no toxic substances.

b Ratio less than 0.8 shows growth inhibiting substances in water sample.

2 For toxic control

$$\frac{\text{colony count per ml Flask F}}{\text{colony count per ml Flask A}} = \text{Ratio}$$

OPTIONAL TEST

3 *For nitrogen and carbon sources that promote growth**

$$\frac{\text{colony count per ml Flask C}}{\text{colony count per ml Flask A}} = \text{Ratio}$$

4 *For nitrogen sources that promote growth**

$$\frac{\text{colony count per ml Flask D}}{\text{colony count per ml Flask A}} = \text{Ratio}$$

5 *For carbon sources that promote bacterial growth**

$$\frac{\text{colony count per ml Flask E}}{\text{colony count per ml Flask A}} = \text{Ratio}$$

G Interpretation of Results:

1 The colony count from Flask A after 20 - 24 hours, at 35°C will depend on the number of organisms initially planted in Flask A and on the strain of A. aerogenes used in the test procedures. This is the reason the control Flask A must be run for each individual series of tests. However, for a given strain of A. aerogenes under identical environmental conditions, the terminal count should be reasonably constant when the initial plant is the same.

Thus, it is essential that the initial colony count on Flask A and Flask B should be approximately equal to secure accurate data.

2 When the ratio exceeds 1.2, it may be assumed that growth stimulating substances are present. However, this procedure is an extremely sensitive test and ratios up to 3.0 would have little significance in actual practice. Therefore, Test C, D, and E do not appear necessary except in special circumstances, when the ratio is between 1.2 and 3.0.

3 Usually Flask C will be very low and flasks D and E will have a ratio of less than 1.2 when the ratio of Flask B/ Flask A is between 0.8 and 1.2. The limiting factors of growth in Flask A are the nitrogen and organic carbon present. An extremely large amount of ammonia nitrogen with no organic carbon could increase the ratio in Flask D above 1.2 or the absence of nitrogen with high carbon concentration could give ratios above 1.2 in Flask E with an A/B ratio between 0.8 and 1.2.

4 A ratio below 0.8 indicates the water contains toxic substances and this ratio includes all allowable tolerances. As indicated in item 2 (above), the 1.2 ratio could go as high as 3.0 without any undesirable results.

5 We are unable to recommend corrective measures in specific cases of defective distillation apparatus. However, a careful inspection of the distillation equipment and a review of production and handling of the distilled water should enable the local laboratory personnel to correct the cause of the difficulty.

*Do not attempt to calculate ratios, 3, 4, or 5 when ratio 1 indicates a toxic reaction.
**Ratio in excess of 1.2 indicates available source for bacterial growth.

CASE EXAMPLES

Test results for various distilled water samples

SOURCE	TEST COUNT	CONTROL COUNT	RATIO	INTERPRETATION
1	< 100	120,000	-----	Toxic Substance
2	74,000	170,000	0.4	Toxic Substance
3	18,000	14,000	1.3	Excellent water
4	21,000	14,000	1.5	Excellent water
5	310,000	60,000	5.2	Growth Substance
6	850,000	37,000	22.9	Growth Substance

REFERENCES

1 Standard Methods for Examination of Water and Wastewater. 12th Edition. 1965. p 578.

2 Geldreich, E. E. and H. F. Clark. Distilled Water Suitability for Microbiological Applications. Journal Milk and Food Technical. In Press. 1965.

This outline was prepared by E. E Geldreich, Chief Bacteriologist, Water Supply Programs Division, WPO, EPA, Cincinnati, OH 45268.

Descriptors: Bacteria, Microbiology, Laboratory, Water Supply, Distillation, Water Quality Control

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Colony Counting on MF (Membrane Filters)

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 40 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: Coverage of this subject will give the participant the background knowledge necessary to recognize and enumerate appropriate colonies and obtain counts per 100 milliliters for the selected membrane.

V. ENTRY LEVEL BEHAVIOR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be able to distinguish between characteristic and uncharacteristic (or background) colonies for the indicator bacteria on their particular selective medium. He/she will select the suitable membrane for counting purposes and obtain the membrane count per 100 milliliters for recording purposes.

B. Conditions: Instructional material as covered in Course Training Manual.

C. Accepted Performance: In attendance to the lecture material covering subject matter.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training Manual Outline: Colony Counting on Membrance Filters

2. 17 slides of X-33 Series: MF Colony Counting

B. Suggested Media:

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review visual and written material and prepare lesson.

2. Sequence slides as desired and place in slide tray.

B. Sequencing:

1. Use of Training Manual Outline information:

a. Introduction

b. Removal of Culture from Incubator

c. Drying Filters before Colony Counts

d. Selection of Acceptable Membrane Filter Culture for Examination

1) Non-Quantitative Tests

2) Quantitative Tests

e. Typical Calculations

2. X-33 Series: MF Colony Counting:

a. Use of Grid System in Counting (X33-1 to X33-2)

b. Stereoscope and fluorescent light orientation (X33-3)

c. Coliform colonies using incandescent light (X33-4)

d. m-ENDO medium showing extreme background growth (X33-5)

e. TNTC (too-numerous-to count) m-ENDO plate (X33-6)

f. m-ENDO medium. Colonies showing light reflection without sheen (X33-7)

g. m-ENDO medium. Confluency and light reflections (X33-8)

h. m-ENDO medium. Confluency (X33-9)

i. m-ENDO medium. Sample volume concentration in portion of working area. (X33-10)

j. m-ENDO medium. Air-bubble under membrane (X33-11)

k. m-ENDO medium. Countable plate (m-ENDO) with good colony distribution (X33-12)

l. MFC medium. Some confluency (X33-13)

m. MFC medium. Good distribution. "x" and "y" indicate background growth. (X33-14)

n. KF Agar. Overloaded TNTC plate (X33-15)

o. KF Agar. TNTC plate with good distribution of colonies (X33-16)

p. KF Agar. Countable plate with good distribution of colonies (X33-17)

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom equipment and supplies:

1. Slide projector and tray

2. Blackboard and chalk

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS:

A. 17 Slides, X-33: Colony Counting on MF

1. Slide X33-1: Effective Filtering Area and Colony Counting Pathway

2. Slide X33-2: Visually Placing Colonies Within Various Grids for Counting Purposes

3. Slide X33-3: Stereoscope and Fluorescent Light Orientation

4. Slide X33-4: Appearance of Typical Coliform Colonies Under Incandescent Light Source

5. Slide X33-5: m-ENDO medium showing too-numerous background growth

6. Slide X33-6: TNTC m-ENDO plate

7. Slide X33-7: m-ENDO plate with examples of light reflection from colonies without sheen

8. Slide X33-8: m-ENDO plate showing examples of confluent colonies and light reflection

9. Slide X33-9: m-ENDO medium showing confluent colonies

10. Slide X33-10: Sample volume concentration in portion of working area

11. Slide X33-11: Air Bubble under Membrane

12. Slide X33-12: Countable Plate (m-ENDO) with Good Colony Distribution

13. Slide X33-13: MFC Medium showing some Colony Confluency

14. Slide X33-14: MFC Medium showing good colony distribution. "x" and "y" indicate background growth.

15. Slide X33-15: KF Agar. Overloaded TNTC plate

16. Slide X33-16: KF Agar. TNTC plate with good distribution of colonies

17. Slide X33-17: KF Agar. Countable plate with good distribution of colonies

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Selection of Sample Filtration Volumes for Membrane Filter Methods

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 30 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: Participant will be ware of the importance and calculation of sample volumes regarding the use of the membrane filter method.

V. ENTRY LEVEL BEHAVIOR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be aware of the relationship of sample volumes to obtaining countable MF plates. He/she will be able to calculate sample volumes when the count/100 ml is known for a particular bacteriological parameter.

B. Conditions: Instructional material as covered in Course Training Manual

C. Accpeted Performance: In attendance to the lecture material covering subject matter.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training Manual Outline: Selection of Sample Filtration Volumes for Membrane Filter Methods.

B. Suggested Media:

1. None required.

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review written material and prepare lesson.

B. Sequencing:

1. A convenient approach to this subject would be to follow the order of presentation as per the outline in the Course Training Manual:

a. Introduction

1) Wide range of filtration volumes

2) Filter limitations with respect to number of colonies

3) Formula for proportionality between colony counts and filtration volumes

b. Bacterial Density

1) Minimum number of colonies

2) Maximum number of colonies

c. Suspended Matter

d. Range of Bacterial Densities Covered by Single-Volume Filtrations

e. Application of a Series of Filtration Volumes

1) Total Coliform

2) Fecal Coliform

3) Fecal Streptococci

f. Selecting Filtration Volumes for Membrane Filter Tests

1) Total Coliform

2) Fecal Coliform

3) Fecal Streptococci

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom Equipment and Supplies

1. Blackboard and chalk

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS:

A. None required.

SUBJECT MATTER: Advance Preparations for Day Three Laboratory

WHEN ACCOMPLISHED: Approximately 1 hour prior to Day Three Laboratory

ACCOMPLISHED BY: Staff

TIME REQUIRED: 1 Hour

PREPARATION REQUIRED	REMARKS
Remove any verification tests from incubator and place at proper postion.	
Remove 72 hour MPN tests from incubators and place each test rack at the proper position.	
Collect sample for MF Testing	Approximately 4 liters, well mixed, and placed in individual sample bottles. Identical sample site as was collected on Day Two
Place 6 KF agar plates/position	KF Agar pre-prepared by staff
Have laboratory operational for laboratory studies	1. MF Test equipment/supplies at each laboratory position 2. Incubators operational 3. MF data sheets distributed

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Laboratory Briefing for Day Three

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 30 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: To ensure understanding of previous laboratory work and to give a briefing for the work to be accomplished during Day Three Laboratory.

V. ENTRY LEVEL BEHAVIOR:

A. Having performed the previous laboratory assignments

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be acquanited with the various areas of instructional material covered in the training course manual and better be able to utilize them in an open book laboratory situation which immediately follows the briefing session.

B. Conditions: Instructional material as covered in various parts of course training manual and as directed by verbal instructions.

C. Accepted Performance: Utilization of assigned pages of course training manual in conjunction with notes taken during briefing sessions.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training Manual Outlines:

Outline	Section	Purpose
MPN Methods	72 hour examination	Provides correct procedural sequence
Verified MF Tests	Verification of Fecal Coliforms	Continuation of verfication procedures
Detailed Membrane Filter Methods	Tests for Fecal Streptococci Group	Provides procedural information
Colony Counting on Membrane Filters	Complete	Provides particulars pn MF colony counting

B. Suggested Media:

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review agenda for time sequencing and written material for content and prepare briefing.

2. Prepare handout (or use blackboard, if desired) indicating to trainee the written material to review. An example of such a handout would be as follows:

Item	Outline and Pages	Standard Methods (14th Edition)
MPN Methods (72 hr procedure)	Examination of Water for Coliforms and Fecal Streptococcus Groups:	
	3-15; 3-18; 3-20; 3-21; 3-24	916-917; 920-921; 922; 943
MF Methods	Detailed Membrane Filter Methods:	
	11-8; 11-9 11-11; 11-12	936-937; 944-945

B. Sequencing:

1. MF Methods (LES Agar techniques and KF Agar techniques)

2. MPN Methods (72 hour test procedures)

3. MF Colony Counting

4. Continue with MPN test rack (the 72 hour examination procedures)

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom Equipment and Supplies:

1. Blackboard and chalk.

2. Demonstration setup

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS:

A. None required.

MPN TEST PROCEDURE :

1. 72 Hour Examination

MF TEST PROCEDURES :

1. Run a given sample for 6 sample volumes and plate out within the 6 LES ENDO agar plates which were prepared on Monday and saved. Use sample volumes which are consistent with Total coliform counts observed during the week as the same sample location will be used for this sample. Do not plate out on the ENDO medium, but, instead, use an LST saturated pad within the cover of the dish. Maintain the plate at 35 C and, just before leaving the lab, transfer to the LES medium and discard the pad.

   LST is lactose broth.

2. Count and record the results of the total coliform MF plates run Tuesday.

3. With a given sample plate out 5 fecal streptococci plates. The plates have been preparepared and are KF Agar. The sample is the same as that which is used with the LES technique above.

   Appropriate samples volumes will be given by instructor.

MF Verfication :

1. Continue verification test.

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Collection and Handling of Bacteriological Samples

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 45 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: Knowledge of this material allows the participant to collect representative and uncontaminated samples to be handled in a manner allowing sample processing which more nearly represents the original sample.

V. ENTRY LEVEL BEHAVIOR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be knowledgeable regarding the current requirements for collection and handling of bacteriological samples as stipulated in Standard Methods for the Examination of Water and Wastewater (Edition lawfully accepted in the Federal Register).

B. Conditions: Instructional material as covered in course training manual and the availability of the current edition of Standard Methods for reference.

C. Accepted Performance: In attendance to the lecture material covering subject material.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training Manual outline: Collection and Handling of Bacteriological Samples

2. Standard Methods (For the 14th Edition: pp. 879; 884-886, 904-906)

3. 14 Slide X38 Series.

B. Suggested Media

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction

1. Review visual and written material and prepare lesson.

2. Sequence slides as desired and place in slide tray.

3. Prepare any handout material, if desired, and arrange to have an

adequate supply prepared.

4. Assemble any desired demonstration equipment/materials and arrange to have them available for the presentation.

B. Sequencing:

1. Classroom presentation of X38

a. Comparison of 12th Edition of Standard Methods to 1934 Ministry of Health (London, England) Slide X38-1.

b. Changes from 12th Edition to 13th Edition of Standard Methods. Slide X38-2.

c. Examples of unacceptable sampling devices. Slides X38-3 to X38-5.

d. Examples of acceptable sampling devices. Slides X38-6 to X38-11.

e. Obtaining grab sample Slide X38-12.

f. Improperly "decanting" sample to obtain air space. Also "hood" separated from stopper. Slide X38-13.

g. Recording data on bottle label. Slide X38-14.

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom Equipment and Supplies.

1. Slide projector and tray.

2. Blackboard and chalk.

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS

A. 14 slides, X-38: Collection and Handling of Bacteriological Samples.

1. Slide X38-1: Printing: Comparison of 12th Edition of Standard Method to 1934 Ministry of Health (London, England).

2. Slide X38-2: Printing: Reservation and storage. Changes from 12th Edition of Standard Methods to the 13th Edition.

3. Slide X38-3: Photo: Spring loaded device.

4. Slide X38-4: Photo: Kemmerrer Sampler.

5. Slide X38-5: Photo: Van Dorn Device.

6. Slide X38-6: Photo: "Lever-type" sampling device in "closed" position.

7. Slide X38-7: Photo: "Lever-type" sampling device in "open" position.

8. Slide X38-8: Drawing: Detail of "Lever-type" sampling device.

9. Slide X38-9: Drawing: Detail of sterile bottle in device.

10. Slide X38-10: Photo: Sterile bottle in model shop made holding device.

11. Slide X38-11: Sterile bag sampler.

12. Slide X38-12: Obtaining a grab sample.

13. Slide X38-13: Erroneous grab sample technique: "decanting sample" to obtain air space and separating hood from stopper.

14. Slide X38-14: Recording data on bottle label.

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Introduction to Statistics and Geometric Means

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 120 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: A basic knowledge of statistics is essential in many survey operations, and, certain calculations, such as the Geometric Means, is necessary in bacteriological report computations.

V. ENTRY LEVEL BEHAVIOR:

A. None required.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be acquainted with the basic terminology of statistics. He/she will have an understanding of the measures of central tendency and be able to calculate the Geometric Means.

B. Conditions: Instructional material as selected by instructor and may consist of handouts, X-T units (slide tape), and verbal instructions.

C. Accepted Performance: In attendance to the classroom session covering subject material.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Handout outline: Statistics for Chemists.

2. X-T Unit (slide tape): Geometric Mean (XT-86) 3 tapes of 35 minutes running time having 78 slides, 3 scripts, and a handout. This unit can be requested on loan for review.

B. Suggested Media:

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Read written material which includes:

a. Outline: Statistics for Chemists

b. XI of this IPW: Description of XT-86 slides

2. Decision required: What media will I use?

   a. None of the available media - proceed to prepare lesson as desired to meet terminal behavior requirement.

   b. Outline only - Prepare required copies and proceed to prepare lesson to meet terminal behavior requirement.

   c. Outline plus XT-86 Series - Prepare required outline copies; Order XT-86 unit from NTOTC; and, after familiarization, prepare lesson as patterned in VIII. B.

B. Sequencing:

1. Distribute copies of handout outline.

2. Utilize handout sequencing to provide statistical basics.

3. Distribute XT-86 handout (single copy is part of unit) to participants (copies must be made prior to session).

4. Initiate programming of XT-86 unit as directed.

   Note: The following information pertains to the XT-86 Geometric Means Unit. See XI for a brief description of the slides.

   Abstract: The instructional objective is to teach the procedure for the calculation of the geometric mean of fecal coliform counts when using logarithms. The viewer may or may not have used logarithms, but should be able to add a series of decimal numbers and divide the total by some whole number. The procedure is illustrated with examples. Sample problems are to be worked by the student after viewing the program. Two checks for gross errors are given.

*For: Persons who have a secondary education or equivalent.

Notes: Time of three tapes is 35 minutes, with 78 slides, 3 scripts and a handout.

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. For each student:

1. Copies of handout material:

   a. Statistics for Chemists

   b. XT-86 handout

*If the participants have less than a secondary education, use XT-85 instead which is intended for those with basic arithmetic skills only

B. For Classroom:

1. Blackboard and chalk

X. IPW REAGENT REQUIREMENTS:

A. None

XI. DESCRIPTION OF VISUAL MATERIALS:

A. XT-86 Geometric Means

Part 1

1. EPA Symbol (XT-86-I-1)
2. Logo (XT-86-I-2)
3. Geometric Mean-Part I (XT-86-I-3)
4. n Coliform Counts (XT-86-I-4)
5. Data Set - Two Counts (XT-86-I-5)
6. Data Set - Three Counts (XT-86-I-6)
7. Importance of n = 3 (XT-86-I-7)
8. Assumption (XT-86-I-8)
9. Definition for Geometric Mean (XT-86-I-9)
10. Definition for Finding Geometric Mean (XT-86-I-10)
11. Finding the Log in 5 Steps (XT-86-I-11)
12. How to Find C (XT-86-I-12)
13. Finding d (XT-86-I-13)
14. Obtaining C (XT-86-I-14)
15. Calculating C (XT-86-I-15)
16. Write the Incomplete (XT-86-I-16)
17. Common Logarithms Using the "Phone Book" (XT-86-I-17)
18. Locating the Mantissa (XT-86-I-18)
19. Examples of M-One Digit (XT-86-I-19)
20. Examples of M-Two Digits (XT-86-I-20)
21. Explaining M Tables (XT-86-I-21)

22. Another Example (XT-86-I-22)

23. Final Example (XT-86-I-23)

24. Summary of Steps - Steps 1 and 2 (XT-86-I-24)

25. Step 3 (XT-86-I-25)

26. Steps 4 and 5 (XT-86-I-26)

27. End of Part I (XT-86-I-27)

28. Clean H_2O (XT-86-I-28)

Part II

1. EPA Symbol (XT-86-II-1)

2. Geometric Mean (XT-86-II-2)

3. GM Formula (XT-86-II-3)

4. Getting Anti-Log in Six Easy Steps (XT-86-II-4)

5. Three Simple Examples (XT-86-II-5)

6. First Step - Determine M (First Example) (XT-86-II-6)

7. Second Step - Locate M on Table (XT-86-II-7)

8. Step Two - Table of Common Logs (XT-86-II-8)

9. Step Two - M = 11394 (XT-86-II-9)

10. Step Three - Determine N (XT-86-II-10)

11. Step Four - Find C (XT-86-II-11)

12. Step Five - Finding d (XT-86-II-12)

13. Step Six - Locate Decimal Point (XT-86-II-13)

14. Step Six - Decimal Point Determined (XT-86-II-14)

15. Second Example - Steps One and Two (XT-86-II-15)

16. Steps Three, Four, Five and Six (XT-86-II-16)

17. Third Example - Steps One and Two (XT-86-II-17)

18. Steps Three, Four, Five and Six (XT-86-II-18)

19. Same Example, Changed Integer (XT-86-II-19)

20. Another Example with M the Same (XT-86-II-20)

21. Summary of Getting Anti-Log (XT-86-II-21)
22. Summary - Steps One and Two (XT-86-II-22)
23. Summary - Steps Three and Four (XT-86-II-23)
24. Summary - Steps Five and Six (XT-86-II-24)
25. End Part II (XT-86-II-25)
26. Clean Water (XT-86-II-26)

Part III

1. EPA Symbol (XT-86-III-1)
2. Part III (XT-86-III-2)
3. Definition (XT-86-III-3)
4. Reference to Part I (XT-86-III-4)
5. Finding the Log in Five Steps (XT-86-III-5)
6. Getting the Anti-Log (XT-86-III-6)
7. First Three Steps (XT-86-III-7)
8. Last 3 Steps (XT-86-III-8)
9. Combining Both Operations for Geometric Mean (XT-86-III-9)
10. Fin the Log Shown in Red (XT-86-III-10)
11. Steps One, Two and Three (XT-86-III-11)
12. Steps Four and Five (XT-86-III-12)
13. Determining the Other Two Logs (XT-86-III-13)
14. Sum of the Three Logs (XT-86-III-14)
15. Quotient (XT-86-III-15)
16. Anti-log of 2.56036 (XT-86-III-16)
17. Geometric Mean of the Three Numbers (XT-86-III-17)
18. Checks for Gross Errors (XT-86-III-18)
19. First Check (XT-86-III-19)
20. Second Check (XT-86-III-20)

21. "Check" Results (XT-86-III-21)

22. End of Part III (XT-86-III-22)

23. Credit Slide (XT-86-III-23)

24. Clean Water (XT-86-III-24)

STATISTICS FOR CHEMISTS

I INTRODUCTION

A Statistics may be defined, for our purpose, as a collection of methods which have been developed for handling numerical data pertaining to samples or portions of entire populations.

B The statistical methods with which we will concern ourselves deal with the presentation and analysis of numerical data from samples.

II FREQUENCY

A Definitions

1 Frequency - indicates how many times a particular score occurs in a collection of data

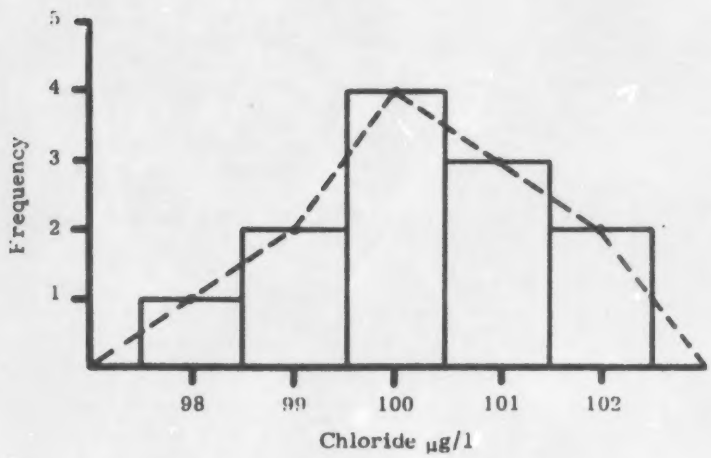
2 Frequency table - a tabular arrangement of data, ranked in ascending or descending order of magnitude, together with the corresponding frequencies

3 Frequency histogram - a set of rectangles having bases on a horizontal axis with centers at the given scores and heights equal to the corresponding frequencies (See Figure 1)

4 Frequency polygon - a line graph of frequencies plotted against scores (can be obtained by connecting midpoints of tops of rectangles in the frequency histogram) (See Figure 1)

Figure 1

Frequency Histogram & Frequency Polygon



B Application

Consider the application of the above definitions to the following set of data, obtained from twelve determinations for chloride in water.

Results (μg/l)		
100	101	99
101	100	100
99	102	100
98	101	102

Table 1

Frequency Table

Chloride (μg/l)	Frequency
98	1
99	2
100	4
101	3
102	2

III MEASURES OF CENTRAL TENDENCY

A Definitions

1 Central tendency - the tendency of values to cluster about a particular value in the distribution

2 Mode - that value which occurs most frequently

3 Median - midpoint of an array of scores. If there is an odd number of observations, n, the median is $X_{\frac{n+1}{2}}$ where $X_{\frac{n+1}{2}}$ represents the $\frac{n+1}{2}$ value in the frequency distribution. If there is an even number of observations the median is $\frac{X_{\frac{n}{2}} + X_{\frac{n}{2}+1}}{2}$, the average of the middle two scores.

4 Mean - arithmetic average of all the values in the sample distribution, denoted by $\bar{X}$. The formula for calculating the sample mean is

$$\bar{X} = \frac{X_1 + X_2 + X_3 \ldots X_n}{n}$$

$$\bar{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

$$\bar{X} = \frac{\Sigma X_i}{n}$$ where there are n number of values.

B Aids in calculation of the mean

Application of the following two statements can reduce errors and amount of time spent in calculating the mean of a distribution.

1 Adding or subtracting a constant to or from each score in a distribution is equivalent to adding or subtracting the same constant to or from the mean of the distribution. Thus the following formula:

$\bar{X}_c = \bar{X} \pm C$ where the X_i's are the values in the distribution with mean $\bar{X}$, and the $X_i \pm C$'s are the values in the distribution with mean $\bar{X}_c$.

2 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the mean of the distribution by the same constant. Thus the following formulas:

(1) $\bar{X}_c = C\bar{X}$

or

(2) $\bar{X}_c = \frac{\bar{X}}{C}$ where the X_i's are the values in the distribution with mean $\bar{X}$,

and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with mean $\bar{X}_c$

C Application

Consider the application of the above definitions to the previously mentioned set of data, obtained from twelve determinations for chloride in water, shown in Table 1.

1 Mode = 100

2 Median = $\frac{X_{\frac{n}{2}} + X_{\frac{n}{2}+1}}{2} = \frac{X_6 + X_7}{2}$

$= \frac{100 + 100}{2} = 100$

3 Mean = $\frac{\Sigma X_i}{n}$

$= \frac{98 + 2\,(99) + 4\,(100) + 3\,(101) + 2\,(102)}{12}$

$= 100.25$

4 Aid in Calculation

Consulting Table 1 and observing that the values are in the neighborhood of 100 we might subtract 100 from each score and obtain the following distribution:

Table 2

Frequency Table

Chloride (μg/l)	Frequency
-2	1
-1	2
0	4
1	3
2	2

Denote the mean of the distribution in Table 1 by $\bar{X}_c$. If we add 100 to each score in the distribution in Table 2, we obtain the scores in the distribution in Table 1; likewise if we add 100 to the mean, X, of the distribution in Table 2, we obtain the mean, $\bar{X}_c$, of the distribution in Table 1.

Thus $\bar{X}_c = \bar{X} + 100$

$$\bar{X}_c = \frac{\Sigma X}{n} + 100$$

$$\bar{X}_c = \frac{1(-2) + 2(-1) + 4(0) + 3(1) + 2(2)}{12} + 100$$

$\bar{X}_c = .25 + 100 = 100.25$

IV MEASURES OF DISPERSION

A Definitions

1 Dispersion - spread or variability of observations in a distribution

2 Range - the difference between the highest value and the lowest value

R = max - min

3 Average deviation - the sum of the deviations of the values from their mean, without regard to sign, divided by the total number of data values (n)

The formula for calculating the average deviation is:

$$d = \frac{\Sigma |X_i - \bar{X}|}{n}$$

4 Average deviation of the mean (D) - the average deviation of individual data items from the mean (d) divided by the square root of the number of data items (n)

The definition of the average deviation of the mean can be expressed by the formula:

$$D = \frac{d}{\sqrt{n}}$$

5 Variance - the sum of the squares of the deviations of the values from their mean divided by the total number of data values (n) minus 1

The definition of the variance can be expressed by the following formula:

$$s^2 = \frac{\Sigma(X_i - \overline{X})^2}{n - 1}$$

6 Standard deviation - the square root of the variance

The definition of the standard deviation can be expressed by the following formula:

$$s = \sqrt{\frac{\Sigma(X_i - \overline{X})^2}{n - 1}}$$

However, the formula commonly used because of its adaptability to the hand calculator is the following:

$$s = \sqrt{\frac{\Sigma X_i^2 - \frac{(\Sigma X_i)^2}{n}}{n - 1}}$$

where there are n number of values.

7 Standard deviation of the mean (S) - the standard deviation of individual data items (s) divided by the square root of the number of data items (n)

The definition of the standard deviation of the mean can be expressed by the formula:

$$S = \frac{s}{\sqrt{n}}$$

8 Relative standard deviation - the standard deviation (s) expressed as a fraction of the mean, $\frac{s}{\overline{X}}$

The relative standard deviation is often expressed as a percent. It is then referred to as the coefficient of variation (V):

$$V = \frac{s}{\overline{X}} \times 100 = \%$$

The relative standard deviation is particularly helpful when comparing the precision of a number of determinations on a given substance at different levels of concentration.

B Aids in Calculation

Application of the following statements can reduce errors and amount of time spent in calculating the variance or standard deviation of a distribution.

1 Adding or subtracting a constant to or from each score in a distribution doesn't affect the variance or standard deviation of the distribution.

Thus the following formulas:

(1) $s_c^2 = s^2$

(2) $s_c = s$

where the X_i's are the values in the distribution with variance s^2 and standard deviation s, and the $X_i \pm C$'s are the values in the distribution with variance s_c^2 and standard deviation s_c.

2 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the variance of that distribution by the square of the same constant.

Thus the following formulas:

(1) $s_c^2 = C^2 s^2$

(2) $s_c^2 = \frac{s^2}{C^2}$

where the X_i's are the values in the distribution with variance s^2, and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with variance s_c^2.

3 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the standard deviation of that distribution by the same constant.

Thus the following formulas:

(1) $s_c = Cs$

or

(2) $s_c = \frac{s}{C}$

where the X_i's are the values in the distribution with standard deviation s, and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with standard deviation s_c.

C Application

Consider the application of the above definitions to the previously mentioned set of data, obtained from twelve determinations for chloride in water, shown in II B, Table 1.

1 Range = 102 - 98 = 4

2 Average deviation - $d = \frac{\Sigma|X_i - \bar{X}|}{n}$

n	X_i	$\lvert X_i - \bar{X}\rvert$	$n\lvert X_i - \bar{X}\rvert$
1	98	2.25	2.25
2	99	1.25	2.50
4	100	.25	1.00
3	101	.75	2.25
2	102	1.75	3.50
	$\bar{X}$ = 100.25		11.50

$$d = \frac{\Sigma|X_i - \bar{X}|}{n} = \frac{11.50}{12} = .96$$

3 Average deviation of the mean -

$$D = \frac{d}{\sqrt{n}}$$

Using calculations from number 2,

$$D = \frac{d}{\sqrt{n}} = \frac{0.96}{\sqrt{12}} = \frac{0.96}{3.46} = 0.28$$

4 Variance - $s^2 = \frac{\Sigma(X_i - \bar{X})^2}{n-1}$

n	X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$	$n(X_i - \bar{X})^2$
1	98	-2.25	5.06	5.06
2	99	-1.25	1.56	3.12
4	100	- .25	.06	.24
3	101	+ .75	.56	1.68
2	102	+1.75	3.06	6.12
				16.22

$$s^2 = \frac{\Sigma(X_i - \bar{X})^2}{n-1} = \frac{16.22}{11} = 1.47$$

5 Standard deviation - $s = \sqrt{\frac{\Sigma X_i^2 - \frac{(\Sigma X_i)^2}{n}}{n-1}}$

n	X_i	nX_i	X_i^2	nX_i^2
1	98	98	9604	9604
2	99	198	9801	19602
4	100	400	10000	40000
3	101	303	10201	30603
2	102	204	10404	20808
		1203		120617

$$s = \sqrt{\frac{120617 - \frac{1203^2}{12}}{11}} = \sqrt{\frac{120617 - 120601}{11}}$$

$$s = \sqrt{\frac{16}{11}} = 1.21$$

6 Aid in calculation

Recalling that adding or subtracting a constant to each score in the distribution doesn't affect the variance or the standard deviation of the distribution we can simplify the computations by first subtracting 100 from each score in the distribution, thus obtaining the frequency distribution shown in Table 2.

n	$X_i - C$	$n(X_i - C)$	$(X_i - C)^2$	$n(X_i - C)^2$
1	-2	-2	4	4
2	-1	-2	1	2
4	0	0	0	0
3	1	3	1	3
2	2	4	4	8
		3		17

$$s^2 = \frac{\Sigma X_i^2 - \frac{(\Sigma X_i)^2}{n}}{n-1}$$

$$s^2 = \frac{17 - \frac{(3)^2}{12}}{11} = \frac{16.25}{11} = 1.48$$

$$s = \sqrt{\frac{\Sigma X_i^2 - \frac{(\Sigma X_i)^2}{n}}{n-1}} = \sqrt{1.48} = 1.22$$

7 Standard deviation of the mean -

$$S = \frac{s}{\sqrt{n}}$$

Using calculations from number 6,

$$S = \frac{s}{\sqrt{n}} = \frac{1.22}{\sqrt{12}} = \frac{1.22}{3.46} = 0.35$$

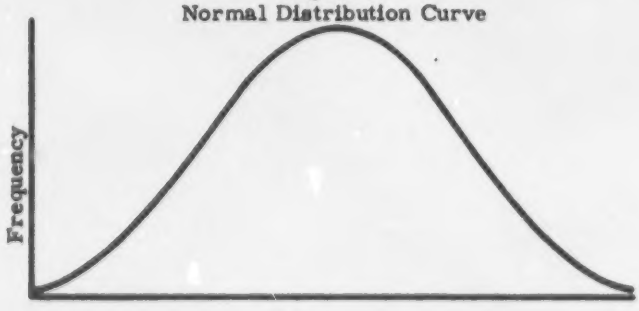
8 Relative standard deviation expressed as a percent (coefficient of variation)

$$V = \frac{s}{\bar{X}} \times 100$$

Using calculations from number 6 for $s = 1.22$ and from number 2 for $\bar{X} = 100.25$,

$$V = \frac{s}{\bar{X}} = \frac{1.22}{100.25} \times 100 = 1.21\%$$

Figure 2
Normal Distribution Curve



V INTRODUCTION TO NORMAL DISTRIBUTION CURVE

A Statistics deals with theoretical curves which are smoother than frequency polygons, obtained from experiments in real life. However, frequency distributions or frequency polygons of experimental data often approximate a mathematical function called the "normal" distribution curve. (See Figure 2)

As shown in Figure 3, the frequency polygon for the 12 determinations for chloride in water is a fairly good approximation of the normal curve. If, however, in the chloride determinations we had obtained 103 instead of 98 and 104 instead of 99 this distribution would not have been a good approximation of the normal curve, as is shown in Figure 4.

Figure 3

Comparison of Normal Curve and Frequency Polygon

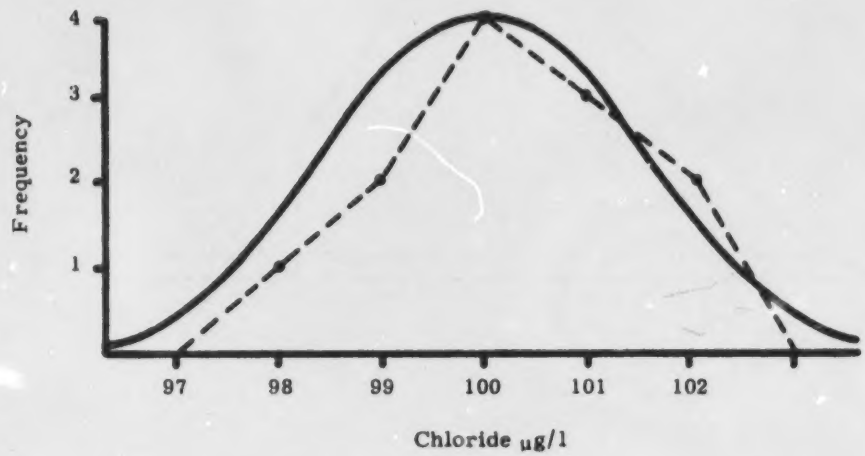
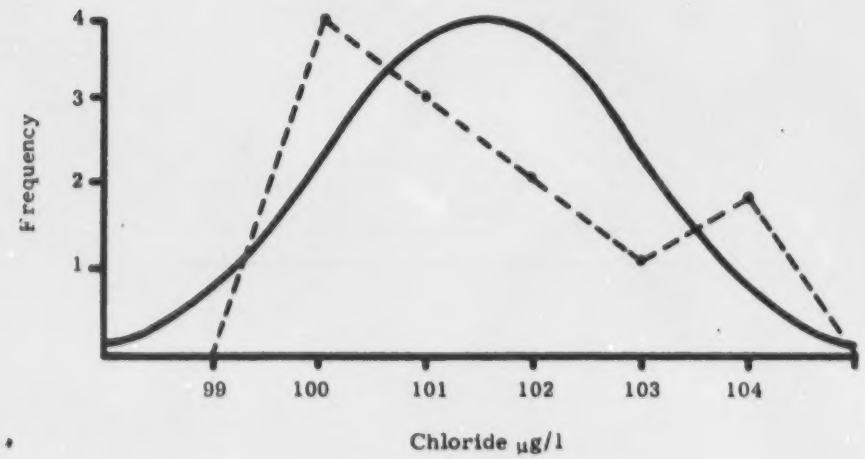


Figure 4

Comparison of Normal Curve and Frequency Polygon



B If a frequency distribution is a good approximation of the normal curve, we can use some facts about the normal curve to give us information about the frequency distribution.

Figure 5 shows the normal distribution in terms of the population mean μ, and the standard deviation of the population σ, and gives the percent of area under the curve between certain points.

Figure 5

Normal Distribution Curve

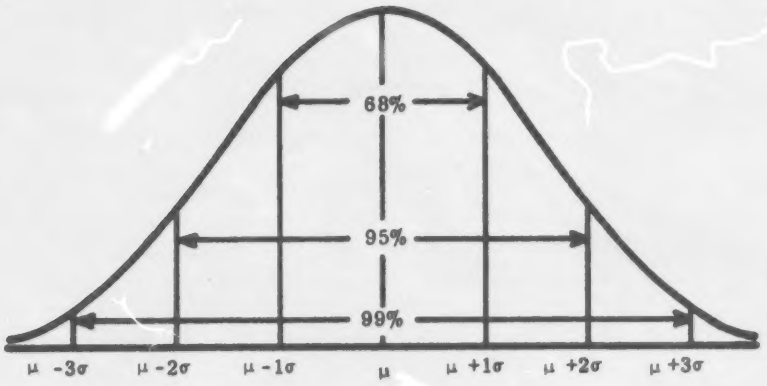
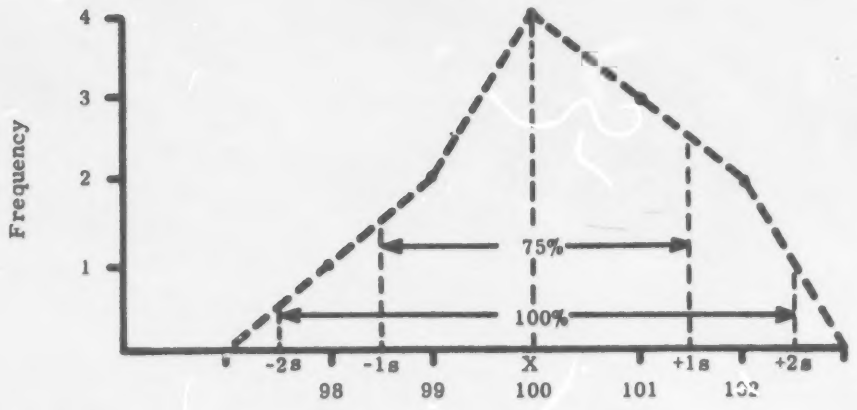


Figure 6

Frequency Distribution Polygon



Chloride μg/l

We may check the distribution of sample data to see if it is a "normal" distribution in the following manner. Substitute the value of the sample mean ($\bar{X}$) for the value of the midline and substitute the value of the sample standard deviation (s) for the limits of the value spans where we might expect certain percentages of the data items to occur. Then we can check the number of data items which actually do occur within these value spans.

Figure 6 demonstrates this application using the chloride data values from Table 1. The data values are marked on the horizontal line and the frequency of the occurrence of each value is marked on the vertical. The midline of the distribution is marked at the value of the sample mean ($\bar{X}$ = 100, See III C 3). The value of the sample standard deviation (s = 1.21, See IV C 5) is used to mark value areas under the curve where different percentages of data values will probably occur. Thus, for the area $\bar{X} \pm 1s$, $\bar{X} - 1s = 98.79$ and $\bar{X} + 1\ s = 101.21$. Therefore, according to the normal distribution curve shown in Figure 5, we might expect about 68% of the data items to have values between 99 and 101. (The values are rounded to whole numbers since the data values are thus recorded).

Consulting Table 1, we find that 75% or 9 of the 12 data items have values in this range. This percentage is shown in Figure 6 by the frequency polygon for the data shown earlier in Figure 3.

Likewise assuming a normal distribution, we would expect 95% of the observations to lie within $\pm 2\sigma$'s from the population mean. In fact, 100% of the observations were within $\pm$ 2 s's from the sample mean.

In both cases the observed percentages are reasonably close to the expected percentages. Other tests exist for determining whether or not a frequency distribution might reasonably be assumed to approximate the normal distribution.

It would be good to become as familiar as possible with the normal distribution since an underlying normal distribution is assumed for many statistical tests of hypothesis.

REFERENCES

1 Bennett, C.A. and Franklin, N.L. Statistical Analysis in Chemistry and the Chemical Industry. John Wiley & Sons, Inc., New York. 1954.

2 Crow, E.L., Davis, F.A., and Maxfield, M.W. Statistics Manual. Dover Publications, Inc., New York. 1960.

3 Dixon, W.J. and Massey, F.J. Introduction to Statistical Analysis. McGraw-Hill Book Co., Inc., New York. 1957.

4 Ostle, B. Statistics in Research. The Iowa State University Press, Iowa. 1963.

5 Youdon, W.J. Statistical Methods for Chemists. John Wiley & Sons, Inc., New York. 1951.

This outline was prepared by L. A. Lederer, Statistician, formerly with Analytical Reference Service, Training Program, NCUIH, SEC. Revised by Audrey E. Donahue, Chemist, National Training Center, Office of Water Programs, EPA. Cincinnati, OH 45268.

SUBJECT MATTER: Advance Preparations for Day Four Laboratory

WHEN ACCOMPLISHED: Approximately 1 hour prior to Day Four Laboratory

ACCOMPLISHED BY: Staff

TIME REQUIRED: 1 Hour

PREPARATION REQUIRED	REMARKS
Remove any remaining MPN tests (96 hour) from the incubators and place at proper positions.	
Remove any remaining verification tests from incubator and place at proper positions	
Collect sample for MF testing	Approximately 4 liters well mixed, diluted with sterile distilled water, and placed in individual sample bottles. Select sample which has a known coliform and fecal coliform count and dilute to give a new count/100 ml which is furnished to class for calculating the CDV. A new source sample could also have been selected which requires no preparation.
Have laboratory operational for laboratory studies	1. MF test equipment and supplies at each laboratory position 2. Incubators operational

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Laboratory Briefing for Day Four

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 45 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: To ensure understanding of previous laboratory work and to give a briefing for the work to be accomplished during Day Four Laboratory.

V. ENTRY LEVEL BEHAVIOR:

A. Having performed the previous laboratory procedures

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be acquainted with the various areas of instructional material covered in the training course manual and better be able to utilize them in an open book laboratory situation which immediately follows the briefing session.

B. Conditions: Instructional material as covered in various parts of course training manual and as directed by verbal instructions.

C. Accepted Performance: Utilization of assigned pages of course training manual in conjunction with notes taken during briefing sessions.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training Manual Outlines:

Outline	Section	Purpose
MPN Methods	96 hour examination	Provides correct procedural sequence
Verified MF Tests	Verification of fecal coliforms	Continuation of Verification procedures
Detailed Membrane Filter Methods	Tests for fecal coliforms	Provides procedural information
Colony Counting on Membrane Filters	Complete	Provides particulars on MF colony counting

B. Suggested Media:

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Review agenda for time sequencing and written material for content and prepare briefing.

2. Prepare handout (or use blackboard, if desired) indicating to trainee the written material to review. An example of such a handout would be as follows:

Item	Outline and Pages	Standard Methods (14th Edition)
MPN Methods (96 hour procedures)	Examination of Water for Coliforms and Fecal Streptococcus Groups:	
	3-15; 3-18; 3-19; 3-21; 3-24	916-917; 920-921; 922; 943
MF Methods	Detailed Membrane Filter Methods:	
	11-10; 11-11	937-939

B. Sequencing:

1. MF Methods (Preparation of MFC broth and the incubation of completed plates)

2. MPN Methods (96 hour test procedures - continue demonstration with the MPN test rack)

3. MF Sample Volumes (Review the calculation of the CDV [Central Dilution Volume] when the count/100 ml is known)

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom Equipment and Supplies:

1. Blackboard and chalk.

2. Demonstration setup

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS:

A. None required.

Day Four Laboratory

MPN TEST PROCEDURE :

1. Complete MPN test procedure if any remains

MF TEST PROCEDURE :

1. Continue Verification Test procedure. If any remaining EC tubes are to be read and recorded this would conclude the Fecal Coliform Verification Test

2. Read and Record the coliform test results of using the enrichment test procedure (LES Agar).

3. Prepare 5 m-ENDO broth plates from the broth which was prepared on Monday and saved in the refrigerator. Discard the remaining broth.

4. Prepare 30ml of MFC broth and from this prepare 6 plates.

Rosolic acid pre-prepared by staff for class use. Plastic bags available for under-water immersion

5. Given information regarding the TC and FC counts/100ml of a sample, calculate the sample volumes to run. Use the following dilution volumes for the respective plates:

TOTAL COLIFORMS

10CDV	5CDV	CDV	1/5CDV	1/10CDV

FECAL COLIFORMS

16CDV	8CDV	CDV	1/8CDV	1/16CDV

CDV refers to Central-Dilution-Volume which is the calculated volume

6. Run the sample and plate on the prepared plates. Incubate at the proper temperatures.

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Post-Course Quiz

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 75 minutes (60 minutes Quiz; 15 minutes review)

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: May be a requirement for course completion.

V. ENTRY LEVEL BEHAVIOR:

A. Attended 95% of course.

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: Receive a critique of the test after collection of same to be aware of correct responses.

B. Conditions: He/she will be given the test and 60 minutes.

C. Accpeted Performance: Obtain at least a 70% test score.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. None required.

B. Suggested Media:

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. Duplicate quiz for number of participants.

2. Prepare overheads of the quiz with answers.

B. Sequencing:

1. Distribute quiz to participants.

2. Allow 60 minutes for completion.

3. Collect quiz - check for name entry as they are collected.

4. Critique quiz by the recommended procedure of use of overhead projection.

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. For each participant:

1. Quiz

B. For instructor:

1. Overhead projection of quiz.

X. IPW REAGENT REQUIREMENTS:

A. None

XI. DESCRIPTION OF VISUAL MATERIALS:

A. See attached example Bacteriological Course Quiz.

Name_____

BACTERIOLOGICAL METHODS IN WATER QUALITY CONTROL PROGRAMS (120.4)

(Date)
(Place)

INDICATE IF THE FOLLOWING ARE TRUE (T) OR FALSE (F):

1. Coliform bacteria ferment lactose with the production of gas.....____
2. Fecal coliform bacteria are effectively separated from the total coliforms by the use of the chemical TTC.....____
3. The elevated temperature test (44.5 C) is useful primarily to separate the coliforms from the fecal streptococci.....____
4. EC medium is primarily used for pathogenic testing.....____
5. Fecal coliforms will also show a golden metallic sheen characteristic on the m-ENDO medium.....____
6. Membrane filters are so effective as filters that they have always proven to be an acceptable bacteriological method.....____
7. Membrane filters have proven to be so tough and durable that unlimited sterilization practices can be used.....____
8. The addition of sodium azide to m-ENDO medium has allowed this medium to effectively differentiate coliforms from background growth.....____
9. MPN tubed media can be stored indefinitely if it is placed in the dark under refrigerated conditions.....____
10. Fecal coliform colonies on MFC medium show a golden metallic-like sheen characteristic.....____
11. The countable plate range for the KF agar plate is from 20 to 80 colonies.....____

Select the best answer from the multiple choices listed: CIRCLE THE ANSWER

12. The countable plate range for the FECAL COLIFORMS on the MFC plate is:

    a. 20-80

    b. not more than 200 of all types

    c. 20-60

    d. 20-100

13. The primary reason for adding alcohol (ethanol) in m-ENDC is:

    a. to keep the coliforms within the countable plate range

    b. allow all colonies to develop good sheen characteristics

    c. prohibit pathogens and fecal streptococci from developing

    d. keep down background growth on the MF

14. Which of the following descriptions is true of the Fecal Coliform medium by the MF method?

    a. use is made of ethanol in preparation; the temperature is elevated; red-hues are typical.

    b. rosolic acid is utilized; TTC is needed in preparation; body temperature is required.

    c. incubation under water is required; a temperature of 44.5 C is manditory; countable colonies have blue colorations.

    d. sodium sulfite is in the formula; lactose is necessary in the formulation; a 35 C temperature of incubation is necessary.

15. With regard to the CONFIRMED TEST by the MPN procedure:

    a. EMB or BGLB may be used; a temperature of 35 C is necessary; loop or inoculation stick transfer may be used; confirmatory tubed medium is held for 48 hours, if necessary.

    b. m-ENDO medium is necessary; gram staining is required; elevated temperatures are necessary; sheen colonies are counted.

    c. all tubes of the presumptive test are transferred to this test; EC medium is the medium of choice; IMViC testing may be required; only positive gas formers are counted.

    d. KF medium is necessary; 35 C is the temperature; broth or agar may be used; 24 hours is the incubation time.

From the given WORD LIST, select the choice which matches the statement on the left. Note the correct response is given for the first statement.

16. Gram Staining Reagent.....____
17. A general term denoting disease organisms.....____
18. The medium most generally used for the confirmed test by the MPN.....____
19. A Coliform bacteria.....____
20. A fecal coliform MPN medium....____
21. The staining reaction for a coliform bacteria.....____
22. The MF medium for Fecal coliforms.....____

WORD LIST

A. Crystal Violet dye
B. Gram Postive organism
C. Gram Negative organism
D. BGLB medium
E. EC medium
F. Escherichia coli
G. Pathogen
H. Cryogen
I. Saphrophyte
J. Salmonella
K. MFC medium
L. LES m-ENDO Medium
M. KF Agar medium
N. Rosolic Acid dye
O. Cryptococcus

Name_____

BACTERIOLOGICAL METHODS IN WATER QUALITY
CONTROL PROGRAMS (120.4)

(Date)
(Place)

INDICATE IF THE FOLLOWING ARE TRUE (T) OR FALSE (F):

1. Coliform bacteria ferment lactose with the production of gas..... T
2. Fecal coliform bacteria are effectively separated from the total coliforms by the use of the chemical TTC..... F
3. The elevated temperature test (44.5 C) is useful primarily to separate the coliforms from the fecal streptococci..... F
4. EC medium is primarily used for pathogenic testing..... F
5. Fecal coliforms will also show a golden metallic sheen characteristic on the m-ENDO medium..... T
6. Membrane filters are so effective as filters that they have always proven to be an acceptable bacteriological method..... F
7. Membrane filters have proven to be so tough and durable that unlimited sterilization practices can be used..... F
8. The addition of sodium azide to m-ENDO medium has allowed this medium to effectively differentiate coliforms from background growth..... F
9. MPN tubed media can be stored indefinitely if it is placed in the dark under refrigerated conditions..... F
10. Fecal coliform colonies on MFC medium show a golden metallic-like sheen characteristic..... F
11. The countable plate range for the KF agar plate is from 20 to 80 colonies..... F

Select the best answer from the multiple choices listed: CIRCLE THE ANSWER

12. The countable plate range for the FECAL COLIFORMS on the MFC plate is:

    a. 20-80

    b. not more than 200 of all types

    (c.) 20-60

    d. 20-100

13. The primary reason for adding alcohol (ethanol) in m-ENDO is:

    a. to keep the coliforms within the countable plate range

    b. allow all colonies to develop good sheen characteristics

    c. prohibit pathogens and fecal streptococci from developing

    (d.) keep down background growth on the MF

14. Which of the following descriptions is true of the Fecal Coliform medium by the MF method?

    a. use is made of ethanol in preparation; the temperature is elevated; red-hues are typical.

    b. rosolic acid is utilized; TTC is needed in preparation; body temperature is required.

    (c.) incubation under water is required; a temperature of 44.5 C is manditory; countable colonies have blue colorations.

    d. sodium sulfite is in the formula; lactose is necessary in the formulation; a 35 C temperature of incubation is necessary.

15. With regard to the CONFIRMED TEST by the MPN procedure:

    (a.) EMB or BGLB may be used; a temperature of 35 C is necessary; loop or inoculation stick transfer may be used; confirmatory tubed medium is held for 48 hours, if necessary.

    b. m-ENDO medium is necessary; gram staining is required; elevated temperatures are necessary; sheen colonies are counted.

    c. all tubes of the presumptive test are transferred to this test; EC medium is the medium of choice; IMViC testing may be required; only positive gas formers are counted.

    d. KF medium is necessary; 35 C is the temperature; broth or agar may be used; 24 hours is the incubation time.

From the given WORD LIST, select the choice which matches the statement on the left. Note the correct response is given for the first statement.

16. Gram Staining Reagent..... A

17. A general term denoting disease organisms..... G

18. The medium most generally used for the confirmed test by the MPN..... D

19. A Coliform bacteria..... F

20. A fecal coliform MPN medium.... E

21. The staining reaction for a coliform bacteria..... C

22. The MF medium for Fecal coliforms..... K

WORD LIST

A. Crystal Violet dye
B. Gram Postive organism
C. Gram Negative organism
D. BGLB medium
E. EC medium
F. Escherichia coli
G. Pathogen
H. Cryogen
I. Saphrophyte
J. Salmonella
K. MFC medium
L. LES m-ENDO Medium
M. KF Agar medium
N. Rosolic Acid dye
O. Cryptococcus

SUBJECT MATTER: Advance Preparations for Day Five Laboratory

WHEN ACCOMPLISHED: 1/2 hour prior to Day Five Laboratory

ACCOMPLISHED BY: Staff

TIME REQUIRED: 1/2 hour

PREPARATION REQUIRED	REMARKS
Remove KF Agar plates from incubator and place at each position	48 hour incubation
Remove MFC plates from incubator and place at each position	
Remove m-ENDO plates from incubator and place at each position	
Have laboratory operational for laboratory studies	MF colony counting equipment

GUIDELINES FOR
INSTRUCTIONAL PACKAGE WORKSHEET

I. SUBJECT MATTER: Laboratory Briefing for Day Five

II. UNIT OF INSTRUCTION: Summary of Topic Presentation

III. ESTIMATED TIME: 10 minutes

IV. JUSTIFICATION FOR THIS INSTRUCTIONAL OBJECTIVE: To give a briefing for the work to be accomplished during Day Five Laboratory.

V. ENTRY LEVEL BEHAVIOR:

A. Having performed the previous laboratory assignments

VI. INSTRUCTIONAL OBJECTIVE:

A. Terminal Behavior: The participant will be acquainted with the various areas of instructional material covered in the training course manual and better be able to utilize them in an open book laboratory situation which immediately follows the briefing session.

B. Conditions: Instructional material as covered in various parts of course training manual and as directed by verbal instructions.

C. Accepted Performance: Utilization of assigned pages of course training manual in conjunction with notes taken during briefing sessions.

VII. INSTRUCTIONAL RESOURCES:

A. Available Media:

1. Training Manual Outline:

Outline	Section	Purpose
Colony Counting on Membrane Filters	Complete	Provides particulars on MF Colony counting

B. Suggested Media:

1. None

VIII. INSTRUCTIONAL APPROACH:

A. Preparation for Instruction:

1. No particular preparation required.

B. Sequencing:

1. Colony Counting - m-ENDO (total coliform)

2. Colony Counting - MFC (fecal coliform)

3. Colony Counting - KF (fecal streptococci)

IX. IPW EQUIPMENT AND SUPPLY REQUIREMENTS:

A. Classroom Equipment and Supplies:

1. Blackboard and chalk

2. Demonstration setup, if desired, of MFC and KF Agar plates.

X. IPW REAGENT REQUIREMENTS:

A. None required.

XI. DESCRIPTION OF VISUAL MATERIALS:

A. None required.

MF TEST PROCEDURE

1. Read and Record m-ENDO plates (total coliform)

2. Read and Record MFC plates (fecal coliform)

3. Read and Record KF Agar plates (fecal streptococci)

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